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(54) Title: GENE EXPRESSION IN BIOLOGICAL CONDITIONS

(57) Abstract: The invention concerns a method of determining the presence or absence of a biological condition in humans, in particular of colon cancer, and of determining the stage of a condition in human tissue by determining an expression pattern of a cell sample. Further, the invention relates to a method of determining the presence or absence of a biological condition in human tissue, and of determining the stage of a biological condition in human tissue, and also for reducing biological abnormalities of a cell suffering from the biological condition. A method for producing antibodies against an expression product of a cell from the tissue is also described. The invention also discloses a pharmaceutical composition for the treatment of a biological condition comprising at least one antibody, and a vaccine for the prophylaxis or treatment of a biological condition. Further the invention describes the use of a method for producing an assay for diagnosing a biological condition in human tissue, the use of a peptide or a gene or a probe for the preparation of a pharmaceutical composition for the treatment of a biological condition in human tissue, and an assay for determining the presence or absence of biological condition in human tissue and for determining an expression pattern of a cell.

Gene expression in biological conditions

Technical field of the invention

The present invention relates to method of determining the presence or absence of a biological condition in animal tissue, wherein the expression of genes in normal tissue and tissue from the biological condition is examined and correlated to standards. The invention further relates to treatment of the biological condition and an assay for determining the condition.

Background

The building of large databases containing human genome sequences is the basis for studies of gene expressions in various tissues during normal physiological and pathologic conditions. Constantly (constitutively) expressed sequences as well as sequences whose expression is altered during disease processes are important for our understanding of cellular properties, and for the identification of candidate genes for future therapeutic intervention. As the number of known genes and ESTs build up in the databases, array-based simultaneous screening of thousands of genes is necessary to obtain a profile of transcriptional behaviour, and to identify key genes that either alone or in combination with other genes, control various aspects of cellular life. One cellular behaviour that has been a mystery for many years is the malignant behaviour of cancer cells. We now know that for example defects in DNA repair can lead to cancer but the cancer-creating mechanism in heterozygous individuals is still largely unknown as is the malignant cell's ability to repeat cell cycles to avoid apoptosis to escape the immune system to invade and metastasize and to escape therapy. There are hints and indications in these areas and excellent progress has been made, buth the myriad of genes interacting with each other in a highly complex multidimensional network is making the road to insight long and contorted.

Similar appearing tumors – morphologically, histochemically, microscopically – can be profoundly different. They can have a different invasive and metastasizing properties, as well as respond differently to therapy. There is thus a need in the art

for methods which distinguish tumors and tissues on different bases than are currently in use in the clinic.

The malignant transformation from normal tissue to cancer is believed to be a multistep process, in which tumorsuppressor genes, that normally repress cancer growth show reduced gene expression and in which other genes that encode tumor promoting proteins (oncogenes) show an increased expression level. Several tumor suppressor genes have been identified up till now, as e.g. p16, Rb, p53 (Nesrin Özören and Wafik S. El-Deiry, Introduction to cancer genes and growth control, In: DNA alterations in cancer, genetic and epigenetic changes, Eaton publishing, Melanie Ehrlich (ed) p. 1-43, 2000.; and references therein). They are usually identified by their lack of expression or their mutation in cancer tissue.

Other examinations have shown this downregulation of transcripts to be partly due to loss of genomic material (loss of heterozygosity), partly to methylation of promotorregions, and partly due to unknown factors (Nesrin Özören and Wafik S. El-Deiry, Introduction to cancer genes and growth control, In: DNA alterations in cancer, genetic and epigenetic changes, Eaton publishing, Melanie Ehrlich (ed) p. 1-43, 2000.; and references therein).

Several oncogenes are known, e.g. cyclinD1/PRAD1/BCL1, FGFs, c-MYC, BCL-2 all of which are genes that are amplified in cancer showing an increased level of transcript (Nesrin Özören and Wafik S. El-Deiry, Introduction to cancer genes and growth control, In: DNA alterations in cancer, genetic and epigenetic changes, Eaton publishing, Melanie Ehrlich (ed) p. 1-43, 2000.; and references therein). Many of these genes are related to cell growth and directs the tumor cells to uninhibited growth. Others may be related to tissue degradation as they e.g. encode enzymes that break down the surrounding connective tissue.

Summary of the invention

In one aspect the present invention relates to a method of determining the presence or absence of a biological condition in animal tissue comprising

collecting a sample comprising cells from the tissue and/or expression products from the cells,

assaying a first expression level of at least one gene from a first gene group, wherein the gene from the first gene group is selected from genes expressed in normal tissue cells in an amount higher than expression in biological condition cells, and/or

assaying a second expression level of at least one gene from a second gene group, wherein the second gene group is selected from genes expressed in a normal tissue cells in an amount lower than expression in biological condition cells,

correlating the first expression level to a standard expression level for normal tissue, and/or the second expression level to a standard expression level for biological condition cells to determine the presence or absence of a biological condition in the animal tissue.

Animal tissue may be tissue from any animal, preferably from a mammal, such as a horse, a cow, a dog, a cat, and more preferably the tissue is human tissue. The biological condition may be any condition exhibiting gene expression different from normal tissue. In particular the biological condition relates to a malignant or premalignant condition, such as a tumor or cancer.

Furthermore, the invention relates to a method of determining the stage of a biological condition in animal tissue,

comprising collecting a sample comprising cells from the tissue,

assaying the expression of at least a first stage gene from a first stage gene group and at least a second stage gene from a second stage gene group, wherein at least one of said genes is expressed in said first stage of the condition in a higher amount than in said second stage, and the other gene is a expressed in said first stage of the condition in a lower amount than in said second stage of the condition,

correlating the expression level of the at least two genes to a standard level of expression determining the stage of the condition.

Thereby, it is possible to determine the biological condition in more details, such as determination of a stage and/or a grade of a tumor.

The methods above may be used for determining single gene expressions, however the invention also relates to a method of determining an expression pattern of a colon cell sample, comprising:

collecting sample comprising colon and/or rectum cells and/or expression products from colon and/or rectum cells,

determining the expression level of two or more genes in the sample, wherein at least one gene belongs to a first group of genes, said gene from the first gene group being expressed in a higher amount in normal tissue than in biological condition cells, and wherein at least one other gene belongs to a second group of genes, said gene from the second gene group being expressed in a lower amount in normal tissue than in biological condition cells, and the difference between the expression level of the first gene group in normal cells and biological condition cells being at least two-fold, obtaining an expression pattern of the colon and/or rectum cell sample.

Gene expression patterns may rely on one or a few genes, but more preferred gene expression patterns relies on expression from multiple genes, whereby a combined information from several genes is obtained.

Further, the invention relates to a method of determining an expression pattern of a colon cell sample independent of the proportion of submucosal, muscle, or connective tissue cells present, comprising:

determining the expression of one or more genes in a sample comprising cells, wherein the one or more genes exclude genes which are expressed in the submucosal, muscle, or connective tissue, whereby a pattern of expression is

formed for the sample which is independent of the proportion of submucosal, muscle, or connective tissue cells in the sample.

The expression pattern may be used in a method according to this information, and accordingly, the invention also relates to a method of determining the presence or absence of a biological condition in human colon and/or rectum tissue comprising,

collecting a sample comprising cells from the tissue,

determining an expression pattern of the cells as defined above,

correlating the determined expression pattern to a standard pattern,

determining the presence or absence of the biological condition is said tissue.

as well as a method for determining the stage of a biological condition in animal tissue, comprising

collecting a sample comprising cells from the tissue,

determining an expression pattern of the cells as defined above,

correlating the determined expression pattern to a standard pattern,

determining the stage of the biological condition is said tissue.

The invention further relates to a method for reducing cell tumorigenicity of a cell, said method comprising

contacting a tumor cell with at least one peptide expressed by at least one gene selected from genes being expressed in an amount two-fold higher in normal cells than the amount expressed in said tumor cell, or

comprising

obtaining at least one gene selected from genes being expressed in an amount two-fold higher in normal cells than the amount expressed in said tumor cell,

introducing said at least one gene into the tumor cell in a manner allowing expression of said gene(s), or

obtaining at least one nucleotide probe capable of hybridising with at least one gene of a tumor cell, said at least one gene being selected from genes being expressed in an amount one-fold lower in normal cells than the amount expressed in said tumor cell, and

introducing said at least one nucleotide probe into the tumor cell in a manner allowing the probe to hybridise to the at least one gene, thereby inhibiting expression of said at least one gene.

In a further aspect the invention relates to a method for producing antibodies against an expression product of a cell from a biological tissue, said method comprising the steps of

obtaining expression product(s) from at least one gene said gene being expressed as defined above,

immunising a mammal with said expression product(s) obtaining antibodies against the expression product.

The antibodies produced may be used for producing a pharmaceutical composition. Further, the invention relates to a vaccine capable of eliciting an immune response against at least one expression product from at least one gene said gene being expressed as defined above.

The invention furthermore relates to the use of any of the methods discussed above for producing an assay for diagnosing a biological condition in animal tissue.

Also, the invention relates to the use of a peptide as defined above as an expression product and/or the use of a gene as defined above and/or the use of a probe as

defined above for preparation of a pharmaceutical composition for the treatment of a biological condition in animal tissue.

In a yet further aspect the invention relates to an assay for determining the presence or absence of a biological condition in animal tissue, comprising

at least one first marker capable of detecting a first expression level of at least one gene from a first gene group, wherein the gene from the first gene group is selected from genes expressed in normal tissue cells in an amount higher than expression in biological condition cells,

at least one second marker capable of detecting a second expression level of at least one gene from a second gene group, wherein the second gene group is selected from genes expressed in normal tissue cells in an amount lower than expression in biological condition cells.

In another aspect the invention relates to an assay for determining an expression pattern of a colon and/or rectum cell, comprising at least a first marker and a second marker, wherein the first marker is capable of detecting a gene from a first gene group as defined above, and the second marker is capable of detecting a gene from a second gene group as defined above.

Detailed description of the invention

Samples

The samples according to the present invention may be any tissue sample, it is however often preferred to conduct the methods according to the invention on epithelial tissue, such as epithelial tissue from the gastro-intestinal tract, in particular form colon and/or rectum. In particular the epithelial tissue may be mucosa.

The sample may be obtained by any suitable manner known to the man skilled in the art, such as a biopsy of the tissue, or a superficial sample scraped from the tissue. The sample may be prepared by forming a cell suspension made from the tissue, or by obtaining an extract from the tissue.

In one embodiment it is preferred that the sample comprises substantially only cells from said tissue, such as substantially only cells from mucosa of the colon-rectum.

Biological condition

The methods according to the invention may be used for determining any biological condition, wherein said condition leads to a change in the expression of at least one gene, and preferably a change in a variety of genes.

Thus, the biological condition may be any malignant or premalignant condition, in particular in colon/rectum, such as an adenocarcinoma, a carcinoma, a teratoma, a sarcoma, and/or a lymphoma.

In relation to the gastro-intestinal tract, the biological condition may also be colitis ulcerosa, Mb. Crohn, diverticulitis, adenomas.

Single gene expression contra expression pattern

The expression level may be determined as single gene approaches, i.e. wherein the determination of expression from one or two or a few genes is conducted. It is preferred that expression from at least one gene from a first (normal) group is determined, said first gene group representing genes being expressed at a higher level in normal tissue, i.e. so-called suppressors, in combination with determination of expression of at least one gene from a second group, said second group representing genes being expressed at a higher level in tissue from the biological condition than in normal tissue, ie. so-called oncogenes. However, determination of the expression of a single gene whether belonging to the first group or second group is within the scope of the present invention. In this case it is preferred that the single gene is selected among genes having a very high change in expression level from normal cells to biological condition cells.

Another approach is determination of an expression pattern from a variety of genes, wherein the determination of the biological condition in the tissue relies on informa-

tion from a variety of gene expression, i.e. rather on the combination of expressed genes than on the information from single genes.

Colorectal tumors

The following data presented herein relates to colorectal tumors, and therefore the description has focused on the gene expression level as one way of identifying genes that lose function in cancer tissue. Genes showing a remarkable downregulation (or complete loss) of the expression level - measured as the mRNA transcript, during the malignant progression in colon from normal mucosa through Dukes A superficial tumors to Dukes B, slightly invasive tumors, to Dukes C that have spread to lymphnodes and finally to Dukes D that have metastasized to other organs, has been examined, as well as genes gaining importance during the differentiation towards malignancy.

Gene groups

The present invention relates to a variety of genes identified either by an EST identification number and/or by a gene identification number. Both type of identification numbers relates to identification numbers of UniGene database, NCBI, build 18.

The various genes have been identified using Affymetrix arrays of the following product numbers:

Human Gene FL array 900 183
HU35K SubA 900 184
HU35K SubB 900 185
HU35K SubC 900 186
HU35K SubD 900 187

First gene group

The first gene group relates to genes being expressed in normal tissue cells in an amount higher than expression in biological condition cells. The term "normal tissue cells" relates to cells from the same type of tissue that is examined with respect to

the biological condition in question. Thus, with respect to colorectal tumors, the normal tissue relates to colorectal tissue, in particular to colorectal mucosa.

The first gene group therefore relates to genes being downregulated in tumors, such genes being expected to serve as tumor suppressor genes, and they are of importance as predictive markers for the disease as loss of one or more of these may signal a poor outcome or an aggressive disease course. Furthermore, they may be important targets for therapy as restoring their expression level, e.g. by gene therapy, may suppress the malignant growth.

For a colorectal tissue sample a gene from the first gene group is preferably selected individually from genes comprising a sequence as identified below by EST

UniGene number		Homologous to
RC_H04768_at		*chrom 15 no homology*
RC_Z39652_at		*Y14593 APM-1 gene adipocyte-specific secretory protein; chrom 1q21.3-q23*
RC_H30270_at		**chrom 18 PAAAA in colon & bladder *no homology***
RC_T47089_s_at		tenascin-X; tenascin-X precursor; unidentified protein
RC_W31906_at		secretagogin; dJ501N12.8 (putative protein) chrom 6
RC_AA279803_at		*chrom 2 no homology*
RC_R01646_at		chrom ***13q32.1-33.3 ; AL159152 ; homology to mouse Pcbp1 - poly(rC)-binding protein 1***
RC_AA099820_at		*BAC clone AC016778*
AA319615_at		secretory carrier membrane protein; secretory carrier membrane protein 2; chrom 15
H07011_at		**tetraspan NET-6 mRNA; transmembrane 4 superfamily; chrom 7**
RC_T68873_f_at		
RC_T40995_f_at		
RC_H81070_f_at		
RC_N30796_at		
RC_W37778_f_at		
RC_R70212_s_at		
RC_AA426330_at		
RC_N33927_s_at		
RC_T90190_s_at		
RC_AA447145_at		
RC_H75860_at		
RC_T71132_s_at		

and from genes comprising a sequence as identified below

"Human chromogranin A ""mRNA,"" complete cds"	**J03915**
Human adipsin/complement factor D "mRNA," complete cds	**M84526**
Homo sapiens MLC-1V/Sb isoform gene	**M24248**
Human aminopeptidase N/CD13 mRNA encoding aminopeptidase "N," complete cds	**M22324**
H.sapiens MT-1l mRNA	**X76717**
H.sapiens GCAP-II gene	**Z70295**
Human somatostatin I gene and flanks	**J00306**
Human YMP "mRNA," complete cds	U52101
H.sapiens mRNA for beta subunit of epithelial amiloride-sensitive sodium channel	X87159
Human K12 protein precursor "mRNA," complete cds	U77643
Human sulfate transporter (DTD) "mRNA," complete cds	U14528
Human transcription factor hGATA-6 "mRNA," complete cds.	U66075
H.sapiens SCAD "gene," exon 1 and joining features	Z80345
Human S-lac lectin L-14-II (LGALS2) gene	M87860
Human mRNA for protein tyrosine phosphatase	D15049
H.sapiens mRNA for tetranectin	X64559
Human 11kd protein "mRNA," complete cds	U28249
Human anti-mullerian hormone type II receptor precursor "gene," complete cds	U29700
Human heparin binding protein (HBp17) "mRNA," complete cds	M60047
Human ADP-ribosylation factor (hARF6) "mRNA," complete cds	M57763
beta -ADD=adducin beta subunit 63 kda isoform/membrane skeleton protein, beta -ADD=adducin beta subunit 63 kda isoform/membrane skeleton protein {alternatively spliced, exon 10 to 13 region} [human, Genomic, 1851 nt, segment 3 of 3].	S81083
Zinc Finger Protein Znf155	HG4243-HT4513
Human glucagon "mRNA," complete cds	J04040
H.sapiens mRNA for hair "keratin," hHb5	X99140
Human tubulin-folding cofactor E "mRNA," complete cds	U61232
Human integrin alpha-3 chain "mRNA," complete cds	M59911
Human NACP gene	U46901
H.sapiens mRNA for flavin-containing monooxygenase 5 (FMO5)	Z47553
Human mRNA for ATF-a transcription factor	X52943
H.sapiens intestinal VIP receptor related protein mRNA	X77777

and and from genes comprising a sequence as identified below

Homo sapiens chromosome 16 BAC clone CIT987SK-	AF001548

815A9 complete sequence.	
Human mRNA for ATP synthase alpha "subunit," complete cds	D14710
Human mRNA for IgG Fc binding "protein," complete cds	D84239
H.sapiens mRNA for carcinoembryonic "antigen," CGM2	X98311
"Homo sapiens (clone lamda-hPEC-3) phosphoenolpyruvate carboxykinase (PCK1) ""mRNA,"" complete cds"	L05144
Human 11-beta-hydroxysteroid dehydrogenase type 2 "mRNA," complete cds	U26726
"Human intestinal mucin (MUC2) ""mRNA,"" complete cds"	L21998
Human mRNA for KIAA0106 "gene," complete cds	D14662
metallothionein	V00594
Human mRNA for IgG Fc binding "protein," complete cds	D84239
H.sapiens mRNA for carcinoembryonic "antigen," CGM2	X98311
"Homo sapiens (clone lamda-hPEC-3) phosphoenolpyruvate carboxykinase (PCK1) ""mRNA,"" complete cds"	L05144
metallothionein	V00594

In a preferred embodiment a gene from the first gene group is preferably selected individually from genes comprising a sequence as identified below by EST

UniGene number		Homologous to
RC_H04768_at		*chrom 15 no homology*
RC_Z39652_at		*Y14593 APM-1 gene adipocyte-specific secretory protein; chrom 1q21.3-q23*
RC_H30270_at		**chrom 18 PAAAA in colon & bladder *no homology***
RC_AA279803_at		*chrom 2 no homology*
RC_R01646_at		chrom ***13q32.1-33.3 ; AL159152 ; homology to mouse Pcbp1 - poly(rC)-binding protein 1***
RC_AA099820_at		*BAC clone AC016778*

and from genes comprising a sequence as identified below

"Human chromogranin A ""mRNA,"" complete cds"	**J03915**
Human adipsin/complement factor D "mRNA," complete cds	**M84526**
Homo sapiens MLC-1V/Sb isoform gene	**M24248**

Human aminopeptidase N/CD13 mRNA encoding aminopeptidase "N," complete cds	**M22324**
H.sapiens MT-1l mRNA	**X76717**
H.sapiens GCAP-II gene	**Z70295**
Human somatostatin I gene and flanks	**J00306**

or selected individually from genes comprising a sequence as identified below by EST

UniGene number Homologous to

RC_H04768_at		*chrom 15 no homology*
RC_Z39652_at		*Y14593 APM-1 gene adipocyte-specific secretory protein; chrom 1q21.3-q23*
RC_H30270_at		**chrom 18 PAAAA in colon & bladder *no homology***
RC_T47089_s_at		tenascin-X; tenascin-X precursor; unidentified protein
RC_W31906_at		secretagogin; dJ501N12.8 (putative protein) chrom 6
RC_AA279803_at		*chrom 2 no homology*
RC_R01646_at		chrom ***13q32.1-33.3 ; AL159152 ; homology to mouse Pcbp1 - poly(rC)-binding protein 1***
RC_AA099820_at		*BAC clone AC016778*
AA319615_at		secretory carrier membrane protein; secretory carrier membrane protein 2; chrom 15
H07011_at		**tetraspan NET-6 mRNA; transmembrane 4 superfamily; chrom 7**

In a more preferred embodiment a gene from the first gene group is selected individually from genes comprising a sequence as identified below by EST

UniGene number Homologous to

RC_H04768_at		*chrom 15 no homology*
RC_Z39652_at		*Y14593 APM-1 gene adipocyte-specific secretory protein; chrom 1q21.3-q23*
RC_H30270_at		**chrom 18 PAAAA in colon & bladder *no homology***
RC_T47089_s_at		tenascin-X; tenascin-X precursor; unidentified protein
RC_W31906_at		secretagogin; dJ501N12.8 (putative protein) chrom 6
RC_AA279803_at		*chrom 2 no homology*
RC_R01646_at		chrom ***13q32.1-33.3 ; AL159152 ; homology to mouse Pcbp1 - poly(rC)-binding protein 1***

AA319615_at		secretory carrier membrane protein; secretory carrier membrane protein 2; chrom 15

In a most preferred embodiment a gene from the first gene group is selected individually from genes comprising a sequence as identified below by EST

UniGene number Homologous to

RC_T47089_s_at		tenascin-X; tenascin-X precursor; unidentified protein
RC_W31906_at		secretagogin; dJ501N12.8 (putative protein) chrom 6
RC_AA279803_at		*chrom 2 no homology*
AA319615_at		secretory carrier membrane protein; secretory carrier membrane protein 2; chrom 15

Second gene group

We have determined genes that are up-regulated (or gained de novo) during the malignant progression of colorectal cancer from normal tissue through Dukes A,B,C and to Dukes D. These genes are potential oncogenes and may be those genes that create or enhance the malignant growth of the cells. The expression level of these genes may serve as predictive markers for the disease course, as a high level may signal an aggressive disease course, and they may serve as targets for therapy, as blocking these genes by e.g. anti-sense therapy, or by biochemical means could inhibit, or slow, the tumor growth. Such up-regulated (or gained de novo) genes, oncogenes, may be classified according to the present invention as genes belonging to second genes group.

With respect to colorectal tumors genes belonging to the second gene group are preferably selected individually from genes comprising a sequence as identified below by EST

UniGene number Homologous to

RC_AA609013_s_at		microsomal dipeptidase (also on 6.8k); chrom 16
RC_AA232508_at		CGI-89 protein; unnamed protein product;

		hypothetical protein
RC_AA428964_at		serine protease-like protease; serine protease homolog=NES1; normal epithelial cell-specific 1
RC_T52813_s_at		dJ28O10.2 (G0S2 (PUTATIVE LYMPHOCYTE G0/G1 SWITCH PROTEIN 2; chrom 1
RC_AA075642_at		gp-340 variant protein; DMBT1/8kb.2 protein
RC_AA007218_at		*chrom 13 no homology*
RC_N33920_at		ubiquitin-like protein FAT10; diubiquitin; dJ271M21.6 (Diubiquitin); chrom 6
RC_N71781_at		KIAA1199 protein, chrom 15
RC_R67275_s_at		alpha-1 (type XI) collagen precursor; collagen, type XI, alpha 1; collagen type XI alpha-1 isoform A; chrom 1
RC_W80763_at		hypothetical protein; chrom 17
RC_AA443793_at		*chrom 7p22 AC006028 BAC clone*
RC_AA034499_s_at		ZNF198 protein; zinc finger protein; FIM protein; Cys-rich protein; zinc finger protein 198; chrom 13
RC_AA035482_at		*chrom 5; AK022505 clone; CalcineurinB (weakly similar)*
RC_AA024482_at		hypothetical protein; unnamed protein product; chrom 17
RC_H93021_at		*chrom 2 ; XM_004890 peptidylprolyl isomerase A (cyclophilin A)*
RC_AA427737_at		*no homology*
RC_AA417078_at		*chrom 7q31; AF017104 clone*
M29873_s_at		**cytochrome P450-IIB (hIIB3) ; 19q13.1-q13.2**
RC_H27498_f_at		
RC_T92363_s_at		
RC_N89910_at		
RC_W60516_at		
RC_AA219699_at		
RC_AA449450_at		

and from genes comprising a sequence as identified below

Homo sapiens (clones "MDP4," MDP7) microsomal dipeptidase (MDP) "mRNA," complete cds	**J05257**
"Homo sapiens reg gene ""homologue,"" complete cds"	**L08010**
H.sapiens mRNA for prepro-alpha2(I) collagen	**Z74616**
"Human S-adenosylhomocysteine hydrolase (AHCY) ""mRNA,"" complete cds"	**M61832**
Transcription Factor IIia	**HG4312-HT4582**
Human gene for melanoma growth stimulatory activity (MGSA)	**X54489**
Human stromelysin-3 mRNA	**X57766**

CDC25Hu2=cdc25+ homolog "[human," "mRNA," 3118 nt]	S78187
Human mRNA for cripto protein	X14253
Human transformation-sensitive protein (IEF SSP 3521) "mRNA," complete cds	**M86752**
Human complement component 2 (C2) gene allele b	L09708
H.sapiens mRNA for ITBA2 protein	X92896
H.sapiens encoding CLA-1 mRNA	Z22555
"Human fibroblast growth factor receptor 4 (FGFR4) ""mRNA,"" complete cds"	L03840
"""Fibronectin,"" Alt. Splice 1"	HG3044-HT3742
tyk2	X54667
Human mRNA for B-myb gene	X13293
"Human phosphofructokinase (PFKM) ""mRNA,"" complete cds"	U24183
Human pre-B cell enhancing factor (PBEF) "mRNA," complete cds	U02020
Human SH2-containing inositol 5-phosphatase (hSHIP) "mRNA," complete cds	U57650
Human interleukin 8 (IL8) "gene," complete cds	**M28130**
"Human lamin B receptor (LBR) ""mRNA,"" complete cds"	L25931
H.sapiens mRNA for protein tyrosine phosphatase	Z48541
Human mRNA for unc-18 "homologue," complete cds	D63851
H.sapiens mRNA for Zn-alpha2-glycoprotein	X59766 Z25521
"Human asparagine synthetase ""mRNA,"" complete cds"	M27396
Human hepatitis delta antigen interacting protein A (dipA) "mRNA," complete cds	U63825
Human splicesomal protein (SAP 61) "mRNA," complete cds	U08815
Human protein kinase C-binding protein RACK7 "mRNA," partial cds	U48251
Human MAC30 "mRNA," 3' end	L19183
Human thrombospondin 2 (THBS2) "mRNA," complete cds	L12350
"Human nicotinamide N-methyltransferase (NNMT) ""mRNA,"" complete cds"	U08021
H.sapiens mRNA for type I interstitial collagenase	X54925
Human cytochrome b561 gene	U29463
Human H19 RNA "gene," complete cds (spliced in silico)	**M32053**
Human collagen type XVIII alpha 1 (COL18A1) "mRNA," partial cds	L22548
Human clone 23733 "mRNA," complete cds.	U79274
Human transforming growth factor-beta induced gene product (BIGH3) "mRNA," complete cds	M77349
"Human breast epithelial antigen BA46 ""mRNA,"" complete cds"	U58516
	X57351
H.sapiens NGAL gene	X99133
Human mRNA for MDNCF (monocyte-derived neutrophil chemotactic factor)	**Y00787**
H.sapiens EF-1delta gene encoding human elongation	Z21507

factor-1-delta	
H.sapiens mRNA for prepro-alpha1(I) collagen	Z74615
Nuclear Factor Nf-Il6	HG3494-HT3688
	U29175
"HNL=neutrophil lipocalin ""[human,"" ovarian cancer cell line ""OC6,"" mRNA ""Partial,"" 534 nt]. /gb=S75256 /ntype=RNA"	S75256

In a preferred embodiment the genes belonging to the second gene group are preferably selected individually from genes comprising a sequence as identified below by EST

UniGene number Homologous to

RC_AA007218_at		*chrom 13 no homology*
RC_AA443793_at		*chrom 7p22 AC006028 BAC clone*
RC_AA035482_at		*chrom 5; AK022505 clone; CalcineurinB (weakly similar)*
RC_H93021_at		*chrom 2 ; XM_004890 peptidylprolyl isomerase A (cyclophilin A)*
RC_AA427737_at		*no homology*
RC_AA417078_at		*chrom 7q31; AF017104 clone*

and from genes comprising a sequence as identified below

In another preferred embodiment genes from the second gene group are selected individually from genes comprising a sequence as identified below

UniGene number Homologous to

RC_AA609013_s_at		microsomal dipeptidase (also on 6.8k); chrom 16
RC_AA232508_at		CGI-89 protein; unnamed protein product; hypothetical protein
RC_AA428964_at		serine protease-like protease; serine protease homolog=NES1; normal epithelial cell-specific 1
RC_T52813_s_at		dJ28O10.2 (G0S2 (PUTATIVE LYMPHOCYTE G0/G1 SWITCH PROTEIN 2; chrom 1
RC_AA075642_at		gp-340 variant protein; DMBT1/8kb.2 protein

RC_AA007218_at		*chrom 13 no homology*
RC_N33920_at		ubiquitin-like protein FAT10; diubiquitin; dJ271M21.6 (Diubiquitin); chrom 6
RC_N71781_at		KIAA1199 protein, chrom 15
RC_R67275_s_at		alpha-1 (type XI) collagen precursor; collagen, type XI, alpha 1; collagen type XI alpha-1 isoform A; chrom 1
RC_W80763_at		hypothetical protein; chrom 17
RC_AA443793_at		*chrom 7p22 AC006028 BAC clone*
RC_AA034499_s_at		ZNF198 protein; zinc finger protein; FIM protein; Cys-rich protein; zinc finger protein 198; chrom 13
RC_AA035482_at		*chrom 5; AK022505 clone; CalcineurinB (weakly similar)*
RC_AA024482_at		hypothetical protein; unnamed protein product; chrom 17
RC_H93021_at		*chrom 2 ; XM_004890 peptidylprolyl isomerase A (cyclophilin A)*
RC_AA427737_at		*no homology*
RC_AA417078_at		*chrom 7q31; AF017104 clone*
M29873_s_at		**Cytochrome P450-IIB (hIIB3) ; 19q13.1-q13.2**

In a more preferred embodiment genes from the second gene group are selected individually from genes comprising a sequence as identified below

5 UniGene number Homologous to

RC_AA609013_s_at		microsomal dipeptidase (also on 6.8k); chrom 16
RC_AA232508_at		CGI-89 protein; unnamed protein product; hypothetical protein
RC_AA428964_at		serine protease-like protease; serine protease homolog=NES1; normal epithelial cell-specific 1
RC_AA075642_at		gp-340 variant protein; DMBT1/8kb.2 protein
RC_AA007218_at		*chrom 13 no homology*
RC_N33920_at		ubiquitin-like protein FAT10; diubiquitin; dJ271M21.6 (Diubiquitin); chrom 6
RC_N71781_at		KIAA1199 protein, chrom 15
RC_R67275_s_at		alpha-1 (type XI) collagen precursor; collagen, type XI, alpha 1; collagen type XI alpha-1 isoform A; chrom 1
RC_W80763_at		hypothetical protein; chrom 17
RC_AA034499_s_at		ZNF198 protein; zinc finger protein; FIM protein; Cys-rich protein; zinc finger protein 198; chrom 13
RC_AA035482_at		*chrom 5; AK022505 clone; CalcineurinB*

		(weakly similar)
RC_AA024482_at		hypothetical protein; unnamed protein product; chrom 17
RC_H93021_at		*chrom 2 ; XM_004890 peptidylprolyl isomerase A (cyclophilin A)*
RC_AA427737_at		*no homology*
RC_AA417078_at		*chrom 7q31; AF017104 clone*
M29873_s_at		**cytochrome P450-IIB (hIIB3) ; 19q13.1-q13.2**

In an even more preferred embodiment genes from the second gene group are selected individually from genes comprising a sequence as identified below

UniGene number Homologous to

RC_AA609013_s_at		microsomal dipeptidase (also on 6.8k); chrom 16
RC_AA007218_at		*chrom 13 no homology*
RC_AA035482_at		*chrom 5; AK022505 clone; CalcineurinB (weakly similar)*
RC_H93021_at		*chrom 2 ; XM_004890 peptidylprolyl isomerase A (cyclophilin A)*
RC_AA427737_at		*no homology*
RC_AA417078_at		*chrom 7q31; AF017104 clone*

such as a sequence as identified below

UniGene number Homologous to

RC_W80763_at		hypothetical protein; chrom 17

The genes from the second gene group discussed above are preferably genes being expressed in all stages of the biological condition, such as all Dukes stages of a colorectal tumor, to be used for determining the biological condition.

Number of genes

As discussed above, it is possible to use a single gene approach determining the expression of one of the genes only, in order to determine the biological condition of the tissue. This is particularly relevant for genes mentioned in the tables in Experiments, since these genes have been determined as having a strong indicativity per

gene. It is however preferred that expression from at least one gene from the first group as well as expression from one gene from the second group is determined to obtain a more statistically significant result, that is more independent of the expression level of the individual gene. In a preferred embodiment expression from more genes from both groups are determined, such as determination of expression from at least two genes from either of the gene groups, such as determination of expression from at least three genes from either of the gene groups, such as determination of expression from at least four genes from either of the gene groups, such as determination of expression from at least five genes from either of the gene groups, such as determination of expression from at least six genes from either of the gene groups, such as determination of expression from at least seven genes from either of the gene groups.

A pattern of characteristic expression of one gene can be useful in characterizing a cell type source or a stage of disease. However, more genes may be usefully analyzed. Useful patterns include expression of at least one, two, three, five, ten, fifteen, twenty, twenty-five, fifty, seventy-five, one hundred or several hundred informative genes.

Expression level

Using the results provided in the accompanying figures and tables, a gene is indicated as being expressed if an intensity value of greater than or equal to 20 is shown. Conversely, an intensity value of less than 20 indicates that the gene is not expressed above background levels. Comparison of an expression pattern to another may score a change from expressed to non-expressed, or the reverse. Alternatively, changes in intensity of expression may be scored, either increases or decreases. Any statistically significant change can be used. Typically changes which are greater than 2-fold are suitable. Changes which are greater than 5-fold are highly significant.

The present invention in particular relates to methods using genes wherein the ratio of the expression level in normal tissue to biological condition tissue for suppressor genes or vice versa of the expression level in biological condition tissue to normal tissue for condition genes is as high as possible, such as at least two-fold change in

expression, such as at least three-fold, such as at least four fold, such as at least five fold, such as at least six fold, such as at least ten fold, such as at least fifteen fold, such as at least twenty fold.

Stages and grades

Stage of a colorectal tumor indicates how deep the tumor has penetrated. Superficial tumors are termed Dukes A and Dukes B and Dukes C are used to describe increasing degrees of penetration into the muscle. The grade of a colorectal tumor is expressed on a scale of I-IV (1-4). The grade reflects the cytological appearance of the cells. Grade I cells are almost normal. Grade II cells are slightly deviant. Grade III cells are clearly abnormal. And Grade IV cells are highly abnormal.

It is important to classify the stage of a cancer disease, as superficial tumors may require a less intensive treatment than invasive tumors. We have therefore used the expression level of genes to identify genes whose expression can be used to identify a certain stage of the disease. We have divided these "Classifiers" into those which can be used to identify Dukes A, B, C, and D stages. We expect that measuring the transcript level of one or more of these genes will lead to a classifier that can add supplementary information to the information obtained from the pathological Dukes classification. For example we believe that gene expression levels that signify a Dukes C will be unfavourable to detect in a Dukes A tumor, as they may signal that the Dukes A tumor has the potential to become a Dukes C tumor. The opposite is probably also true, that an expression level that signify Dukes A will be favorable to have in a Dukes C tumor. In that way independent information may be obtained from Dukes pathological classification and a classification based on gene expression levels is made.

Thus, in one embodiment the invention relates to a method as described above further comprising the steps of determining the stage of a biological condition in the animal tissue, comprising assaying a third expression level of at least one gene from a third gene group, wherein a gene from said second gene group, in one stage, is expressed differently from a gene from said third gene group.

In another aspect the invention relates to method of determining the stage of a biological condition in animal tissue,

comprising collecting a sample comprising cells from the tissue,

assaying the expression of at least a first stage gene from a first stage gene group and/or at least a second stage gene from a second stage gene group, wherein at least one of said genes is expressed in said first stage of the condition in a higher amount than in said second stage, and the other gene is a expressed in said first stage of the condition in a lower amount than in said second stage of the condition,

correlating the expression level of the assessed genes to a standard level of expression determining the stage of the condition.

The method of determining the stage of a tumor may be combined with determination of the biological condition or may be an independent method as such. The difference in expression level of a gene from one stage to the expression level of the gene in another group is preferably at least two-fold, such as at least three-fold.

Thus, the invention relates to a method of determining the stage of a colorectal tumor, wherein the stage is selected from colon cancer stages Dukes A, Dukes B, Dukes C, and Dukes D, comprising assaying at least the expression of Dukes A stage gene from a Dukes A stage gene group, at least one Dukes B stage gene from a Dukes B stage gene group, at least the expression of Dukes C stage gene from a Dukes C stage gene group, and/or at least one Dukes D stage gene from a Dukes D stage gene group, wherein at least one gene from each gene group is expressed in a significantly different amount in that stage than in one of the other stages.

The genes selected may be a gene from each gene group being expressed in a significantly higher amount in that stage than in one of the other stages, such as:

a Dukes A stage gene selected individually from any gene comprising a sequence as identified below as EST

RC_AA599199_at		ALU seq.
RC_R12694_at		**unnamed protein product BAA91641, chrom 10**
RC_H91325_s_at		**aldolase B; aldolase B (aa 1-364); chrom 9**
RC_N51709_at		chrom X
RC_N72610_at		-
RC_N69263_at		*chrom 10; AK026414 clone (only 108 nt hom)*
RC_T15817_f_at		iNOS, inducible nitric oxide synthase

RC_F03077_f	**chromosome 17, clone hRPC.15**
RC_AA599199	**Alu seq**
RC_AA207015	**clone RP4-733M16 on chromosome 1p36.11-36.23**
RC_AA234916	**chromosome 19 clone CTC-461H2**
RC_N92239_a	**Wnt inhibitory factor-1 (WIF-1), chromosome 12**
RC_N93958_s	**phospholipase A2, group X (PLA2G10),**
U95301_at	**phospholipase A2, group X (PLA2G10),**
RC_AA426330	**chromosome 17, clone hRPC.1110_E_20**
RC_AA024658	**clone SCb-254N2 (UWGC:rg254N02) from 6p21**
RC_H88540_a	**heat shock protein 90, 1q21.2-q22**

or any gene comprising a sequence as identified below

5

D87444_at	Human mRNA for KIAA0255 "gene," complete cds
U18291_at	Human CDC16Hs "mRNA," complete cds
L76568_xpt3_f_at	S26 from Homo sapiens excision and cross link repair protein (ERCC4) "gene," complete genomic sequence. /gb=L76568 /ntype=DNA /annot=exon
U45328_s_at	"Human ubiquitin-conjugating enzyme (UBE2I) ""mRNA,"" complete cds"
Z14982_rna1_at	H.sapiens gene for major histocompatibility complex encoded proteasome subunit LMP7.
AD000092_cds7_s_at	RAD23A gene (human RAD23A homolog) extracted from Homo sapiens DNA from chromosome 19p13.2 cosmids "R31240," R30272 and R28549 containing the "EKLF," "GCDH," "CRTC," and RAD23A "genes," genomic sequence
D86973_at	Human mRNA for KIAA0219 "gene," partial cds
X81636_at	H.sapiens clathrin light chain a gene

M59916_at	Human acid sphingomyelinase (ASM) "mRNA," complete cds
X85781_s_at	"H.sapiens NOS2 ""gene,"" exon 27 /gb=X85781 /ntype=DNA /annot=exon"
M57731_s_at	"Human gro-beta ""mRNA,"" complete cds"
U49188_at	Human placenta (Diff33) "mRNA," complete cds
X53800_s_at	Human mRNA for macrophage inflammatory protein-2beta (MIP2beta)
U56816_at	Human kinase Myt1 (Myt1) "mRNA," complete cds.
HG1067-HT1067_r_at	Mucin (Gb:M22406)

Human migration inhibitory factor-related protein 8 (MRP8) "gene," complete cds	M21005
Human acyloxyacyl hydrolase "mRNA," complete cds	M62840
Human PEP19 (PCP4) "mRNA," complete cds	U52969
H.sapiens Humig mRNA	X72755
H.sapiens PISSLRE mRNA	X78342
H.sapiens mRNA for twist "protein," partial. /gb=Y11180 /ntype=RNA	Y11180
Human mRNA for TGF-beta superfamily "protein," complete cds	AB000584
Human mRNA for "MSS1," complete cds	D11094
Human complement factor B "mRNA," complete cds	L15702
"Homo sapiens GTP-binding protein (RAB2) ""mRNA,"" complete cds"	M28213
Human translational initiation factor 2 beta subunit (eIF-2-beta) "mRNA," complete cds	M29536
Human E16 "mRNA," complete cds	M80244
IEX-1=radiation-inducible immediate-early gene "[human," "placenta," mRNA "Partial," 1223 nt]	S81914
Human CDC16Hs "mRNA," complete cds	U18291
Human DD96 "mRNA," complete cds	U21049
Human (memc) "mRNA," 3'UTR. /gb=U30999 /ntype=RNA	U30999
"Human ubiquitin-conjugating enzyme (UBE2I) ""mRNA,"" complete cds"	U45328
"Human fetal brain glycogen phosphorylase B ""mRNA,"" complete cds"	U47025
"Human BTG2 (BTG2) ""mRNA,"" complete cds"	U72649
Human jun-B mRNA for JUN-B protein	X51345
Human chaperonin 10 "mRNA," complete cds	U07550
H.sapiens RING4 cDNA	X57522
H.sapiens genes TAP1, TAP2, LMP2, LMP7 and DOB.	X66401
H.sapiens mRNA for alpha 4 protein	Y08915
Homo sapiens interleukin-1 receptor-associated kinase (IRAK) "mRNA," complete cds	L76191
"Human von Willebrand factor ""mRNA,"" 3' end"	M10321
Human chromosome segregation gene homolog CAS "mRNA," complete cds	U33286
Human Bruton's tyrosine kinase-associated protein-135 "mRNA," complete cds.	U77948
"Human KH type splicing regulatory protein KSRP ""mRNA,"" complete cds."	U94832
H.sapiens ADE2H1 mRNA showing homologies to SAICAR	X53793

synthetase and AIR carboxylase of the purine pathway (EC "6.3.2.6," EC 4.1.1.21)	

a Dukes B stage gene is selected individually from any gene comprising a sequence as identified below

RC_T67463_s_at		cathepsin O2; X; K
RC_W94688_at		perilipin
RC_AA126743_at		*Z97200 PAC chrom 1q24; PMX1 homeobox gene*
RC_AA236547_at		*no homology*
RC_AA255567_at		angiopoietin-related protein-2; angiopoietin-like 2
RC_AA421256_at		-
RC_AA386386_s_at	PPPPP	-
RC_AA452549_at	PPPPP	PRO1659; hypothetical protein chrom 11

M63262_at	5-lipoxygenase activating protein (FLAP), 13q12
R67290_at	Interleukine 14
N36619_at	
L19161_at	translation initiation factor 2, subunit 3", Xp22.2-22.1
RC_AA496035	Chromosome 1? (TIGR)
L29217_s_at	CDC-like kinase 3 (CLK3), 15q24
RC_W73194_a	Dermatoponin, 1q12-q23
RC_N69507_a	hypothetical protein PRO1847 (Alu according to TIGR)
RC_H15814_s	adipose most abundant gene transcript 1
M84526_at	D component of complement (adipsin)

or any gene comprising a sequence as identified below

U57316_at	Human GCN5 (hGCN5) "gene," complete cds
X66839_at	H.sapiens MaTu MN mRNA for p54/58N protein
J04599_at	Human hPGI mRNA encoding bone small proteoglycan I "(biglycan)," complete cds
X57579_s_at	H.sapiens activin beta-A subunit (exon 2)
J02874_at	Human adipocyte lipid-binding "protein," complete cds
M11749_at	Human Thy-1 glycoprotein "gene," complete cds
U06863_at	Human follistatin-related protein precursor "mRNA," complete cds
U51010_s_at	"Human nicotinamide N-methyltransferase ""gene,"" exon 1 and 5' flanking region. /gb=U51010 /ntype=DNA /annot=exon"
U08021_at	"Human nicotinamide N-methyltransferase (NNMT) ""mRNA,"" complete cds"
HG3044-HT3742_s_at	"""Fibronectin,"" Alt. Splice 1"

X02761_s_at Human mRNA for fibronectin (FN precursor)
X02544_at Human mRNA for alpha1-acid glycoprotein (orosomucoid)
M62505_at Human C5a anaphylatoxin receptor "mRNA," complete cds
J05070_at Human type IV collagenase "mRNA," complete cds
U16306_at Human chondroitin sulfate proteoglycan versican V0 splice-variant precursor peptide "mRNA," complete cds
M14218_at Human argininosuccinate lyase "mRNA," complete cds
L77567_s_at "Homo sapiens mitochondrial citrate transport protein (CTP) ""mRNA,"" 3' end"
M63391_rna1_at Human desmin gene, complete cds.
D13643_at Human mRNA for KIAA0018 "gene," complete cds
D79985_at Human mRNA for KIAA0163 "gene," complete cds

Human adipocyte lipid-binding "protein," complete cds	J02874
Human A1 protein "mRNA," complete cds	U29680
Human LGN protein "mRNA," complete cds	U54999
Human skeletal muscle LIM-protein SLIM2 "mRNA," partial cds	U60116
Human mRNA for alpha1-acid glycoprotein (orosomucoid)	X02544
Human mRNA for fibronectin receptor alpha subunit	X06256
H.sapiens P1-Cdc21 mRNA	X74794
H.sapiens mRNA for fibulin-2	X82494
H.sapiens 5T4 gene for 5T4 Oncofetal antigen	Z29083
Homo sapiens mRNA for osteoblast specific factor 2 (OSF-2os)	D13666
Mac25	HG987-HT987
"Human lysozyme ""mRNA,"" complete cds with an Alu repeat in the 3' flank"	J03801
Human metalloproteinase (HME) "mRNA," complete cds	L23808
Human alpha-1 collagen type IV gene, exon 52.	M26576
Human lumican "mRNA," complete cds	U21128
Human mRNA for fibronectin (FN precursor)	X02761
Human mRNA fragment for elongation factor TU (N-terminus). /gb=X03689 /ntype=RNA	X03689
Human mRNA for type IV collagen alpha -2 chain	X05610
Human mRNA for collagen VI alpha-1 C-terminal globular domain	X15880
"H.sapiens," gene for Membrane cofactor protein	X59405
H.sapiens SOD-2 gene for manganese superoxide dismutase. /gb=X65965 /ntype=DNA /annot=exon	X65965
H.sapiens NMB mRNA	X76534
H.sapiens vimentin gene	Z19554
Human chaperonin 10 "mRNA," complete cds	U07550
H.sapiens RING4 cDNA	X57522
H.sapiens genes TAP1, TAP2, LMP2, LMP7 and DOB.	X66401
H.sapiens mRNA for alpha 4 protein	Y08915
Homo sapiens interleukin-1 receptor-associated kinase (IRAK) "mRNA," complete cds	L76191
"Human von Willebrand factor ""mRNA,"" 3' end"	M10321
Human chromosome segregation gene homolog CAS "mRNA," complete cds	U33286

Human Bruton's tyrosine kinase-associated protein-135 "mRNA," complete cds.	U77948
"Human KH type splicing regulatory protein KSRP ""mRNA,"" complete cds."	U94832
H.sapiens ADE2H1 mRNA showing homologies to SAICAR synthetase and AIR carboxylase of the purine pathway (EC "6.3.2.6," EC 4.1.1.21)	X53793
"""Globin,"" Beta"	HG1428-HT1428
"Human alpha-1 collagen type I ""gene,"" 3' end"	M55998
H.sapiens mRNA for SOX-4 protein	X70683
"Human mRNA for collagen binding protein ""2,"" complete cds"	D83174
Human SPARC/osteonectin "mRNA," complete cds	J03040
Human PRAD1 mRNA for cyclin	X59798

a Dukes C stage gene is selected individually from any gene comprising a sequence as identified below

RC_D45556_at		*chrom 15; AL390085 clone*
RC_W86214_at		
RC_AA039439_s_at		*novel gene KIAA0134 protein 19q13.3*
RC_AA128935_at		
RC_AA134158_s_at		class I homeodomain; homeo-box protein, chrom 7
RC_AA232646_at		chrom 17, *AF266756 sphingo-sine kinase (SPHK1*
RC_AA401184_at		*no homology*
RC_AA436840_at		
RC_AA488655_at		
RC_AA181902_at	PPPPP	*AC007201 on chrom 19 (only 80nt hom)*

RC_AA122350	chromosome 8
AA374109_at	spondin 2, extracellular matrix protein, chromosome 4
RC_AA621755	transcription factor Dp-2, 3q23
RC_AA442069	sodium channel 2, 12q12
RC_T40767_a	chromosome 19
RC_AA488655	Mus?
RC_AA398908	
RC_AA447764	hypothetical protein, chromosome 4
RC_N69136_a	

or any gene comprising a sequence as identified below

M20681_at Human glucose transporter-like protein-III "(GLUT3)," complete cds

D50914_at	Human mRNA for KIAA0124 "gene," partial cds
L37362_at	Homo sapiens (clone d2-115) kappa opioid receptor (OPRK1) "mRNA," complete cds
X66114_rna1_at	H.sapiens gene for 2-oxoglutarate carrier protein.
M32053_at	Human H19 RNA "gene," complete cds (spliced in silico)
Y00787_s_at	Human mRNA for MDNCF (monocyte-derived neutrophil chemotactic factor)
U64444_at	Human ubiquitin fusion-degradation protein (UFD1L) "mRNA," complete cds
X95325_s_at	H.sapiens mRNA for DNA binding protein A variant
X02419_rna1_s_at	H.sapiens uPA gene
X57522_at	H.sapiens RING4 cDNA
AB001325_at	Human AQP3 gene for aquaporine 3 (water "channel)," partail cds
AB002315_at	Human mRNA for KIAA0317 "gene," complete cds. /gb=AB002315 /ntype=RNA
L12760_s_at	"Human phosphoenolpyruvate carboxykinase (PCK1) ""gene,"" complete cds with repeats"
M80899_at	Human novel protein AHNAK "mRNA," partial sequence

Ribosomal Protein L39 Homolog	HG2874-HT3018
Homo sapiens (clone d2-115) kappa opioid receptor (OPRK1) "mRNA," complete cds	L37362
Human kell blood group protein mRNA	M64934
	U73167
Human cancellous bone osteoblast mRNA for serin protease with IGF-binding "motif," complete cds	D87258-
Human interferon-inducible protein 27-Sep "mRNA," complete cds	J04164
"Human sickle cell beta-globin ""mRNA,"" complete cds"	M25079
	M29277
"Human spermidine synthase ""mRNA,"" complete cds"	M34338
Human copine I "mRNA," complete cds	U83246
"""Globin,"" Beta"	HG1428-HT1428
"Human alpha-1 collagen type I ""gene,"" 3' end"	M55998
H.sapiens mRNA for SOX-4 protein	X70683
"Human mRNA for collagen binding protein ""2,"" complete cds"	D83174
Human SPARC/osteonectin "mRNA," complete cds	J03040
Human PRAD1 mRNA for cyclin	X59798

a Dukes D stage gene is selected individually from any gene comprising a sequence as identified below

RC_N91920_at	AAAAP	*chrom 16p12-p11.2 ; XN_007994 retinoblastoma binding protein*

RC_AA621601_at	AAAAP	*chrom 17 XM_009868 RAB36 ARS oncogene family*
RC_AA121433	**Axin, chromosome 16**	
RC_N91920_a	**RB protein binding protein, chromosome 16**	
RC_AA621601	**GTP-binding protein Rab36, chromosome 17**	
RC_AA454020	**NADPH quinone oxidoreductase homolog; p53 induced, chromosome 2**	
RC_Z39652_a	**APM-1 gene, chromosome 18**	

or any gene comprising a sequence as identified below

X17644_s_at	Human GST1-Hs mRNA for GTP-binding protein
Y12812_at	H.sapiens RFXAP mRNA
X60486_at	H.sapiens H4/g gene for H4 histone
X52221_at	H.sapiens ERCC2 "gene," exons 1 & 2 (partial)
L06175_at	Homo Sapiens P5-1 "mRNA," complete cds
Z48481_at	H.sapiens mRNA for membrane-type matrix metalloproteinase 1
X54232_at	Human mRNA for heparan sulfate proteaglycan (glypican)
L08010_at	"Homo sapiens reg gene ""homologue,"" complete cds"
L27706_at	Human chaperonin protein (Tcp20) gene complete cds
L15533_rna1_at	Homo sapiens pancreatits-associated protein (PAP) gene, complete cds.
X51408_at	Human mRNA for n-chimaerin
K02765_at	Human complement component C3 "mRNA," alpha and beta "subunits," complete cds
U38904_at	Human zinc finger protein C2H2-25 "mRNA," complete cds

Homo sapiens FRG1 "mRNA," complete cds	L76159
Human cyclin protein "gene," complete cds	M15796
Human U2 small nuclear RNA-associated B" antigen "mRNA," complete cds	M15841
Human mRNA export protein Rae1 (RAE1) "mRNA," complete cds.	U84720
Human protease-activated receptor 3 (PAR3) "mRNA," complete cds.	U92971
H.sapiens mRNA for mediator of receptor-induced toxicity	X84709
H.sapiens RFXAP mRNA	Y12812
Human mRNA for "Qip1," complete cds	AB002533
Human mRNA for transferrin receptor	X01060
"metastasis-associated gene ""[human,"" highly metastatic lung cell subline ""Anip[937],"" mRNA ""Partial,"" 978 nt]"	S79219

The genes selected may be a gene from each gene group being expressed in a significantly lower amount in that stage than in one of the other stages, such as:

a Dukes A stage gene is selected individually from any gene comprising a sequence as identified below

RC_N32411_f_at	PAPPP	Myc-associated zinc-finger protein of human islet; chrom 16
RC_AA243858_at	PAPPP	KIAA0882 protein
RC_AA486283_at	PAPPP	ras-like protein; ras-related C3 botulinum toxin substrate; dJ20J23
RC_AA490930_at	PAPPP	chrom 18; *KIAA1468 protein*
RC_H54088_s_at	PPPPP	ribosomal protein L41
RC_H59052_f_at	PPPPP	fungal sterol-C5-desaturase homolog; ORF; thymosin beta-4
RC_R49198_s_at	PPPPP	-
RC_T73572_f_at	PPPPP	ferritin L-chain; L apoferritin
RC_AA477483_at	PPPPP	no matching est

or any gene comprising a sequence as identified below

Homo sapiens SKB1Hs "mRNA," complete cds. /gb=AF015913 /ntype=RNA	AF015913
Mucin (Gb:M22406)	HG1067-HT1067
Human platelet activating factor "acetylhydrolase," brain "isoform," 45 kDa subunit (LIS1) gene	U72342
Homosapiens ERK activator kinase (MEK2) mRNA	L11285
Human 20-kDa myosin light chain (MLC-2) "mRNA," complete cds	J02854
H.sapiens lysosomal acid phosphatase gene (EC 3.1.3.2) Exon 1 (and joined CDS).	X15525
Human mRNA for matrix Gla protein	**X53331**
H.sapiens mRNA for diacylglycerol kinase	X62535
Human heat shock protein (hsp 70) gene, complete cds.	M11717
Human TRPM-2 protein gene	**M63379**

a Dukes B stage gene is selected individually from any gene comprising a sequence as identified below

RC_D59847_at	PPAPP	proSAAS; granin-like neuroendocrine peptide precursor
RC_F05038_at	PPAPP	polyamine modulated factor-1; polyamine modulated factor 1
RC_N41059_at	PPAPP	chrom 3
RC_T23460_at	PPAPP	chrom 3; *IFNAR2 21q22.11*
RC_W42789_at	PPAPP	chrom 8 *AF268037 C8ORF4 protein (C8ORF4) chrom 8 ORF*
RC_AA460017_i_at	PPAPP	*BAC clone chrom 16*
RC_AA482127_at	PPAPP	KIAA1142 protein
RC_AA504806_at	PPAPP	*chrom 2 AF052107 clone 23620 mRNA sequence*
RC_T90037_at	PPPPP	unnamed protein product, chrom 4
RC_AA432130_at	PPPPP	KIAA0867 protein, chrom 12

or any gene comprising a sequence as identified below

Human gene for mitochondrial acetoacetyl-CoA thiolase	D10511
Human mRNA for transcription factor "AREB6," complete cds	D15050
Human mRNA for KIAA0248 "gene," partial cds	D87435
Homo sapiens (clone CC6) NADH-ubiquinone oxidoreductase subunit "mRNA," 3' end cds	L04490
Human phosphoglucomutase 1 (PGM1) "mRNA," complete cds	M83088
Homo sapiens guanylin "mRNA," complete cds	**M97496**
"Human trans-Golgi p230 ""mRNA,"" complete cds"	U41740
H.sapiens mRNA for vacuolar proton "ATPase," subunit D	X71490
H.sapiens mRNA for 3-hydroxy-3-methylglutaryl coenzyme A synthase	X83618
Human mRNA for KIAA0018 "gene," complete cds	D13643
"Mucin ""1,"" ""Epithelial,"" Alt. Splice 9"	HG371-HT26388
H.sapiens mRNA for L-3-hydroxyacyl-CoA dehydrogenase	X96752

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a Dukes C stage gene is selected individually from any gene comprising a sequence as identified below

RC_N30231_at	PPPAP	Lsm4 protein; U6 snRNA-associated Sm-like protein

		LSm4; glycine-rich protein
RC_W73790_f_at	PPPAP	immunoglobulin-related protein 14.1; lambda L-chain C region; omega protein, chrom 22
RC_AA412184_at	PPPAP	*chrom 1p36; d89060 dolichyl-diphosphooligosaccharide-protein glycosyltransferase*
RC_AA521303_at	PPPAP	methionine adenosyltransferase regulatory beta subunit; dTDP-4-keto-6-deoxy-D-glucose 4-reductase, chrom 5
RC_AA461174_at	PPPPP	*8p21.3-p22 AB020860 anti-oncogene*
AA393432_s_at	PPPPP	chrom 2, Unknown; unnamed protein product AAD20029

or any gene comprising a sequence as identified below

Homo sapiens colon mucosa-associated (DRA) "mRNA," complete cds	**L02785**
Human Ig J chain gene	**M12759**
Human selenium-binding protein (hSBP) "mRNA," complete cds. /gb=U29091 /ntype=RNA	**U29091**
H.sapiens mRNA for sigma 3B protein	**X99459**
Human ERK1 mRNA for protein serine/threonine kinase	**X60188**
Human mRNA for mitochondrial 3-oxoacyl-CoA "thiolase," complete cds	**D16294**
"Biliary ""Glycoprotein,"" Alt. Splice ""5,"" A"	HG2850-HT4814
Human AQP3 gene for aquaporine 3 (water "channel)," partail cds	AB001325
Human CD14 mRNA for myelid cell-specific leucine-rich glycoprotein	X13334
Human thioredoxin "mRNA," nuclear gene encoding mitochondrial "protein," complete cds	U78678
Human mitochondrial ATPase coupling factor 6 subunit (ATP5A) "mRNA," complete cds	M37104
"Human MHC class II HLA-DP light chain ""mRNA,"" complete cds"	M57466
Human mRNA for early growth response protein 1 (hEGR1)	X52541
Human mRNA for mitochondrial 3-ketoacyl-CoA thiolase beta-subunit of trifunctional "protein," complete cds	D16481
Homo sapiens laminin-related protein (LamA3) "mRNA," complete cds	L34155
H.sapiens mRNA for selenoprotein P	Z11793
Human hkf-1 "mRNA," complete cds	D76444
Homo sapiens nuclear domain 10 protein (ndp52) "mRNA," complete cds	U22897

Human X104 "mRNA," complete cds	L27476
H. sapiens cDNA for RFG	X77548
H.sapiens mRNA for Progression Associated Protein	Y07909
Human liver "2,4-dienoyl-CoA" reductase "mRNA," complete cds	U49352
Human A33 antigen precursor "mRNA," complete cds	U79725
H.sapiens pS2 protein gene	X52003
Human RASF-A PLA2 "mRNA," complete cds	M22430
Homo sapiens pstI mRNA for pancreatic secretory inhibitor (expressed in neoplastic tissue).	Y00705
Human CO-029	M35252

a Dukes D stage gene is selected individually from any gene comprising a sequence as identified below

RC_R72886_s_at	PPPPA	KIAA0422; adenylyl cyclase type VI, chrom 12
RC_AA026030_at	PPPPA	chrom 1
RC_Z39006_at	PPPPA	hypothetical protein, chrom 17
RC_AA435908_at	PPPPA	*chrom 19; ac011491 clone and 20 nt hom. RAB2, RAS oncogene family*
RC_AA057829_s_at	PPPPA	growth-arrest-specific protein; growth arrest-specific 6; AXL stimulatory factor, chrom 13
RC_R72087_at	PPPPA	*chrom 5 EST; hom to chrom 20 AL356652 clone*
RC_H04242_at	PPPPA	ras related protein Rab5b; RAB5B, member RAS oncogene family
RC_R97304_f_at	PPPPA	HLA-drb5; cell surface glycoprotein; MHC HLA-DR-beta chain precursor chrom 6
RC_N48609_at	PPPPA	*chrom 11; AC004584 chrom 17*
RC_W86850_f_at	PPPPA	*chrom 22 ? X96924 mitochondrial citrate tranbsport region*
RC_AA130603_at	PPPPA	*ak024908 clone*
RC_AA479610_at	PPPPA	*singleton ak025344 clone*
RC_AA490593_i_at	PPPPA	*chrom 17 ? Synaptobrevin2 (VAMP2) AF135372*
RC_AA054321_s_at	PPPPA	*6p21 HLA class i region; AC004202 clone*

RC_D60328_at	PPPP P	chrom 6, unknown; ring finger protein 5
RC_H96850_at	PPPP P	*oligosaccharyltransferase d89060 1p36.1 (also C-class)*
RC_AA127444_at	PPPP P	chrom 1 *no homology*
RC_AA242824_at	PPPP P	*chrom 11; ac005233 PAC clone chrom 22*
AA405775_s_at	PPPP P	similar to CAA16821 (PID:g3255952)

or any gene comprising a sequence as identified below

Human complement component C3 "mRNA," alpha and beta "subunits," complete cds	K02765
H.sapiens mRNA for adenosine "triphosphatase," calcium	Z69881
Human skeletal muscle LIM-protein SLIM1 "mRNA," complete cds	U60115
Human platelet-derived growth factor receptor alpha (PDGFRA) "mRNA," complete cds	M21574
Human mRNA for KIAA0247 "gene," complete cds	D87434
Human mRNA for KIAA0171 "gene," complete cds	D79993
Human Down syndrome critical region protein (DSCR1) "mRNA," complete cds	U28833
Human Ki nuclear autoantigen "mRNA," complete cds	U11292

Expression patterns

The objects of the invention are achieved by providing one or more of the embodiments described below. In one embodiment a method is provided of determining an expression pattern of a cell sample preferably independent of the proportion of submucosal, muscle and connective tissue cells present. Expression is determined of one or more genes in a sample comprising cells, said genes being selected from the same genes as discussed above and shown in the tables of the Examples.

It is an object of the present that characteristic patterns of expression of genes can be used to characterize different types of tissue. Thus, for example gene expression patterns can be used to characterize stages and grades of colorectal tumors. Similarly, gene expression patterns can be sued to distinguish cells having a colorectal origin from other cells. Moreover, gene expression of cells which routinely contaminate colorectal tumor biopsies has been identified, and such gene

expression can be removed or subtracted from patterns obtained from colorectal biopsies. Further, the gene expression patterns of single-cell solutions of colorectal tumor cells have been found to be far freer of interfering expression of contaminating muscle, submucosal, and connective tissue cells that biopsy samples.

The one or more genes exclude genes which are expressed in the submucosal, muscle, and connective tissue. A pattern of expression is formed for the sample which is independent of the proportion of submucosal, muscle, and connective tissue cells in the sample.

In another aspect of the invention a method of determining an expression pattern of a cell sample is provided. Expression is determined of one or more genes in a sample comprising cells. A first pattern of expression is thereby formed for the sample. Genes which are expressed in submucosal, muscle, and connective tissue cells are removed from the first pattern of expression, forming a second pattern of expression which is independent of the proportion of submucosal, muscle, and connective tissue cells in the sample.

Another embodiment of the invention provides a method for determining an expression pattern of a colorectal mucosa or colorectal cancer cell. Expression is determined of one or more genes in a sample comprising colorectal mucosa or colorectal cancer cells; the expression determined forms a first pattern of expression. A second pattern of expression which was formed using the one or more genes and a sample comprising predominantly submucosal, muscle, and connective tissue cells, is subtracted from the first pattern of expression, forming a third pattern of expression. The third pattern of expression reflects expression of the colorectal mucosa or colorectal cancer cells independent of the proportion of submucosal, muscle, and connective tissue cells present in the sample.

Diagnosing

In another embodiment of the invention a method is provided of detecting an invasive tumor in a patient. A marker is detected in a sample of a body fluid. The body fluid is selected from the group consisting of blood, plasma, serum, faeces,

mucus, sputum, cerebrospinal fluid and/or urine. The marker is an mRNA or protein expression product of a gene which is more prevalent in submucosal, muscle, and connective tissue than in the body fluid. An increased amount of the marker in the body fluid indicates a tumor which has become invasive in the patient.

In another aspect of the invention a method is provided for diagnosing a colorectal cancer. A first pattern of expression is determined of one or more genes in a colonic tissue sample suspected of being neoplastic. The first pattern of expression is compared to a second and third reference pattern of expression. The second pattern is of the one or more genes in normal colorectal mucosa and the third pattern is of the one or more genes in colorectal cancer. A first pattern of expression which is found to be more similar to the third pattern than the second indicates neoplasia of the colorectal tissue sample.

According to yet another aspect of the invention a method is provided for predicting outcome or prescribing treatment of a colorectal tumor. A first pattern of expression is determined of one or more genes in a colorectal tumor sample. The first pattern is compared to one or more reference patterns of expression determined for colorectal tumors at a grade between I and IV. The reference pattern which shares maximum similarity with the first pattern is identified. The outcome or treatment appropriate for the grade of tumor of the reference pattern with the maximum similarity is assigned to the colorecteal tumor sample.

In another embodiment of the invention a method is provided for determining grade of a colorecteal tumor. A first pattern of expression is determined of one or more genes in a colorectal tumor sample. The first pattern is compared to one or more reference patterns of expression determined for colorectal tumors at a grade between I and IV. The grade of the reference pattern with the maximum similarity is assigned to the colorecteal tumor sample.

Yet another embodiment of the invention provides a method to determine stage of a colorectal tumor as described above. A first pattern of expression is determined of one or more genes in a colorectal tumor sample. The first pattern is compared to one or more reference patterns of expression determined for colorectal tumors at different stages. The reference pattern which shares maximum similarity with the

first pattern is identified. The stage of the reference pattern with the maximum similarity is assigned to the colorecteal tumor sample.

In still another embodiment of the invention a method is provided for identifying a tissue sample as colo-rectal. A first pattern of expression is determined of one or more genes in a tissue sample. The first pattern is compared to a second pattern of expression determined obtained for normal mucosa cells. Similarity between the first and the second patterns suggests that the tissue sample is mucosa in its origin. This method being particularly useful when diagnosing metastasis possibly distant from its origin.

Another aspect of the invention is a method to aid in diagnosing, predicting outcome, or prescribing treatment of a colorectal cancer. A first pattern of expression is determined of one or more genes in a first colorectal tissue sample. A second pattern of expression is determined of the one or more genes in a second colorectal tissue sample. The first colorectal tissue sample is a normal colorectal mucosa sample or an earlier stage or lover grade of colorectal tumor than the second colorectal tissue sample. The first pattern of expression is compared to the second pattern of expression to identify a first set of genes which are increased in the second colorectal tissue sample relative to the first colorectal tissue sample and a second set of genes which are decreased in the second colorectal tissue sample relative to the first colorectal tissue sample. Those genes which are expressed in submucosal, muscle or connective tissue are removed from the first set of genes. Those genes which are not expressed in submucosal, muscle, or connective tissue are removed from the second set of genes.

Independence of submucosal, muscle and connective tissue

Since a biopsy of the tissue often contains more tissue material, than the tissue to be examined, such as connective tissue, when the tissue to be examined is epithelial or mucosa, the invention also relates to methods, wherein the expression pattern of the tissue is independent of the amount of connective tissue in the sample.

Biopsies contain epithelial cells that most often are the targets for the studies, and in addition many other cells that contaminate the epithelial cell fraction to a varying extent. The contaminants include histiocytes, endothelial cells, leukocytes, nerve cells, muscle cells etc. Micro dissection is the method of choice for DNA examination, but in case of expression studies this procedure is difficult due to RNA degradation during the procedure. The epithelium may be gently removed and the expression in the remaining submucosa and underlying connective tissue (the colon wall) monitored. Genes expressed at high or low levels in the colon wall should be interrogated when performing expression monitoring of the mucosa and tumors. A similar approach could be used for studies of epithelia in other organs.

Normal mucosa lining the colon lumen from colons for colon cancer was scraped off. Then biopsies were taken from the denuded submucosa and connective tissue, reaching approximately 5 mm into the colon wall, and immediately disintegrated in guanidinium isothiocyanate. Total RNA may be extracted, pooled, and poly(A)$^+$ mRNA may be prepared from the pool followed by conversion to double-stranded cDNA and in vitro transcription into cRNA containing biotin-labeled CTP and UTP.

Genes that are expressed and genes that are not expressed in colon wall can both interfere with the interpretation of the expression in a biopsy, and should be interrogated when interpreting expression intensities in tumor biopsies, as the colon wall component of a biopsy varies in amount from biopsy to biopsy.

When having determined the pattern of genes expressed in colon wall components said pattern may be subtracted from a pattern obtained from the sample resulting in a third pattern related to the mucosa (epithelial) cells.

In another aspect of the invention a method is provided for determining an expression pattern of a colorectal tissue sample independent of the proportion of submucosal, muscle and connective tissue cells present. A single-cell suspension of disaggregated colorectal tumor cells is isolated from a colorectal tissue sample comprising colorectal tumor cells is isolated form a coloretal tissue sample comprising colorectal cells, submucosal cells, muscle cells, and connective tissue cells. A pattern of expression is thus formed for the sample which is independent of

the proportion of submucosal, muscle, and connective tissue cells in the colorectal tissue sample.

Yet another method relates to elimination mRNA from colon wall components before determining the pattern, e.g. by filtration and/or affinity chromatography to remove mRNA related to the colon wall.

Detection

Working with human tumor material requires biopsies, and working with RNA requires freshly frozen or immediately processed biopsies. Apart from the cancer tissue, biopsies do inevitably contain many different cell types, such as cells present in the blood, connective and muscle tissue, endothelium etc. In the case of DNA studies, microdissection or laser capture are method of choice, however the time.dependent degradation of RNA makes it difficult to perform manipulation of the tissue for more than a few minutes. Furthermore, studies of expressed sequences may be difficult on the few cells obtained via microdissection or laser capture, as these may have an expression pattern that deviates from the predominant pattern in a tumor due to large intratumoral heterogeneity.

In the present context high density expression arrays may be used to evaluate the impact of colorectal wall components in colorectal tumor biopsies, and tested preparation of single cell solutions as a means of eliminating the contaminants. The results of these evaluations permit us to design methods of evaluating colorectal samples without the interfering background noise caused by ubiquitous contaminating submucosal, muscle, and connective tissue cells. The evaluating assays of the invention may be of any type.

While high density expression arrays can be used, other techniques are also contemplated. These include other techniques for assaying for specific mRNA species, including RT-PCR and Northern Blotting, as well as techniques for assaying for particular protein products, such as ELISA, Western blotting, and enzyme assays. Gene expression patterns according to the present invention are determined by measuring any gene product of a particular gene, including mRNA and protein. A pattern may be for one or more gene.

RNA or protein can be isolated and assayed from a test sample using any techniques known in the art. They can for example be isolated from fresh or frozen biopsy, from formalin-fixed tissue, from body fluids, such as blood, plasma, serum, urine, or sputum.

The data provided of expression for submucosal, muscle, and connective tissue can be used in at least three ways to improve the quality of data for a tested sample. The genes identified in the data as expressed can be excluded from the testing or from the analysis. Alternatively, the intensity of expression of the genes expressed in the submucosal, muscle, and connective tissue can be subtracted from the intensity of expression determined for the tests tissue.

The data collected and disclosed here as "connective tissue" is presumed to contain both muscle and submucosal gene expression as well. Thus it represents the composite expression of these cell types which can typically contaminate a colorectal biopsy.

Detection of expression

Expression of genes may in general be detected by either detecting mRNA from the cells and/or detecting expression products, such as peptides and proteins.

mRNA detection

The detection of mRNA of the invention may be a tool for determining the developmental stage of a cell type may be definable by its pattern of expression of messenger RNA. For example, in particular stages of cells, high levels of ribosomal RNA are found whereas relatively low levels of other types of messenger RNAs may be found. Where a pattern is shown to be characteristic of a stage, a stage may be defined by that particular pattern of messenger RNA expression. The mRNA population is a good determinant of developmental stage, will be correlated with other structural features of the cell. In this manner, cells at specific developmental stages will be characterized by the intracellular environment, as well as the extracellular environment. The present invention also allows the combination of

definitions based, in part, upon antigens and, in part, upon mRNA expression. In one embodiment, the two may be combined in a single incubation step. A particular incubation condition may be found which is compatible with both hybridization recognition and non-hybridization recognition molecules. Thus, e.g., an incubation condition may be selected which allows both specificity of antibody binding and specificity of nucleic acid hybridization. This allows simultaneous performance of both types of interactions on a single matrix. Again, where developmental mRNA patterns are correlated with structural features, or with probes which are able to hybridize to intracellular mRNA populations, a cell sorter may be used to sort specifically those cells having desired mRNA population patterns.

It is within the general scope of the present invention to provide methods for the detection of mRNA. Such methods often involve sample extraction, PCR amplification, nucleic acid fragmentation and labeling, extension reactions, transcription reactions and the like.

Sample preparation

The nucleic acid (either genomic DNA or mRNA) may be isolated from the sample according to any of a number of methods well known to those of skill in the art. One of skill will appreciate that where alterations in the copy number of a gene are to be detected genomic DNA is preferably isolated. Conversely, where expression levels of a gene or genes are to be detected, preferably RNA (mRNA) is isolated.

Methods of isolating total mRNA are well known to those of skill in the art. In one embodiment, the total nucleic acid is isolated from a given sample using, for example, an acid guanidinium-phenol-chloroform extraction method and polyA.sup.+ mRNA is isolated by oligo dT column chromatography or by using (dT)n magnetic beads (see, e.g., Sambrook et al., Molecular Cloning: A Laboratory Manual (2nd ed.), Vols. 1-3, Cold Spring Harbor Laboratory, (1989), or Current Protocols in Molecular Biology, F. Ausubel et al., ed. Greene Publishing and Wiley-Interscience, New York (1987)).

The sample may be from tissue and/or body fluids, as defined elsewhere herein. Before analyzing the sample, e.g., on an oligonucleotide array, it will often be

desirable to perform one or more sample preparation operations upon the sample. Typically, these sample preparation operations will include such manipulations as extraction of intracellular material, e.g., nucleic acids from whole cell samples, viruses and the like, amplification of nucleic acids, fragmentation, transcription, labeling and/or extension reactions. One or more of these various operations may be readily incorporated into the device of the present invention.

DNA Extraction

DNA extraction may be relevant in case possible mutations in the genes are to be dtermined in addition to the determination of expression of the genes.

For those embodiments where whole cells, or other tissue samples are being analyzed, it will typically be necessary to extract the nucleic acids from the cells or viruses, prior to continuing with the various sample preparation operations. Accordingly, following sample collection, nucleic acids may be liberated from the collected cells, viral coat, etc., into a crude extract, followed by additional treatments to prepare the sample for subsequent operations, e.g., denaturation of contaminating (DNA binding) proteins, purification, filtration, desalting, and the like.

Liberation of nucleic acids from the sample cells, and denaturation of DNA binding proteins may generally be performed by physical or chemical methods. For example, chemical methods generally employ lysing agents to disrupt the cells and extract the nucleic acids from the cells, followed by treatment of the extract with chaotropic salts such as guanidinium isothiocyanate or urea to denature any contaminating and potentially interfering proteins.

Alternatively, physical methods may be used to extract the nucleic acids and denature DNA binding proteins, such as physical protrusions within microchannels or sharp edged particles piercing cell membranes and extract their contents. Combinations of such structures with piezoelectric elements for agitation can provide suitable shear forces for lysis.

More traditional methods of cell extraction may also be used, e.g., employing a channel with restricted cross-sectional dimension which causes cell lysis when the

sample is passed through the channel with sufficient flow pressure. Alternatively, cell extraction and denaturing of contaminating proteins may be carried out by applying an alternating electrical current to the sample. More specifically, the sample of cells is flowed through a microtubular array while an alternating electric current is applied across the fluid flow. Subjecting cells to ultrasonic agitation, or forcing cells through microgeometry apertures, thereby subjecting the cells to high shear stress resulting in rupture are also possible extraction methods.

Filtration

Following extraction, it will often be desirable to separate the nucleic acids from other elements of the crude extract, e.g., denatured proteins, cell membrane particles, salts, and the like. Removal of particulate matter is generally accomplished by filtration, flocculation or the like. Further, where chemical denaturing methods are used, it may be desirable to desalt the sample prior to proceeding to the next step. Desalting of the sample, and isolation of the nucleic acid may generally be carried out in a single step, e.g., by binding the nucleic acids to a solid phase and washing away the contaminating salts or performing gel filtration chromatography on the sample, passing salts through dialysis membranes, and the like. Suitable solid supports for nucleic acid binding include, e.g., diatomaceous earth, silica (i.e., glass wool), or the like. Suitable gel exclusion media, also well known in the art, may also be readily incorporated into the devices of the present invention, and is commercially available from, e.g., Pharmacia and Sigma Chemical.

Alternatively, desalting methods may generally take advantage of the high electrophoretic mobility and negative of DNA compared to other elements. Electrophoretic methods may also be utilized in the purification of nucleic acids from other cell contaminants and debris. Upon application of an appropriate electric field, the nucleic acids present in the sample will migrate toward the positive electrode and become trapped on the capture membrane. Sample impurities remaining free of the membrane are then washed away by applying an appropriate fluid flow. Upon reversal of the voltage, the nucleic acids are released from the membrane in a substantially purer form. Further, coarse filters may also be overlaid on the barriers to avoid any fouling of the barriers by particulate matter, proteins or nucleic acids,

thereby permitting repeated use.

Separation of contaminants by chromatography

In a similar aspect, the high electrophoretic mobility of nucleic acids with their negative charges, may be utilized to separate nucleic acids from contaminants by utilizing a short column of a gel or other appropriate matrix or gel which will slow or retard the flow of other contaminants while allowing the faster nucleic acids to pass.

This invention provides nucleic acid affinity matrices that bear a large number of different nucleic acid affinity ligands allowing the simultaneous selection and removal of a large number of preselected nucleic acids from the sample. Methods of producing such affinity matrices are also provided. In general the methods involve the steps of a) providing a nucleic acid amplification template array comprising a surface to which are attached at least 50 oligonucleotides having different nucleic acid sequences, and wherein each different oligonucleotide is localized in a predetermined region of said surface, the density of said oligonucleotides is greater than about 60 different oligonucleotides per 1 cm.sup.2, and all of said different oligonucleotides have an identical terminal 3' nucleic acid sequence and an identical terminal 5' nucleic acid sequence. b) amplifying said multiplicity of oligonucleotides to provide a pool of amplified nucleic acids; and c) attaching the pool of nucleic acids to a solid support.

For example, nucleic acid affinity chromatography is based on the tendency of complementary, single-stranded nucleic acids to form a double-stranded or duplex structure through complementary base pairing. A nucleic acid (either DNA or RNA) can easily be attached to a solid substrate (matrix) where it acts as an immobilized ligand that interacts with and forms duplexes with complementary nucleic acids present in a solution contacted to the immobilized ligand. Unbound components can be washed away from the bound complex to either provide a solution lacking the target molecules bound to the affinity column, or to provide the isolated target molecules themselves. The nucleic acids captured in a hybrid duplex can be separated and released from the affinity matrix by denaturation either through heat, adjustment of salt concentration, or the use of a destabilizing agent such as formamide, TWEEN.TM.-20 denaturing agent, or sodium dodecyl sulfate (SDS).

Affinity columns (matrices) are typically used either to isolate a single nucleic acid typically by providing a single species of affinity ligand. Alternatively, affinity columns bearing a single affinity ligand (e.g. oligo dt columns) have been used to isolate a multiplicity of nucleic acids where the nucleic acids all share a common sequence (e.g. a polyA).

Affinity matrices

The type of affinity matrix used depends on the purpose of the analysis. For example, where it is desired to analyze mRNA expression levels of particular genes in a complex nucleic acid sample (e.g., total mRNA) it is often desirable to eliminate nucleic acids produced by genes that are constitutively overexpressed and thereby tend to mask gene products expressed at characteristically lower levels. Thus, in one embodiment, the affinity matrix can be used to remove a number of preselected gene products (e.g., actin, GAPDH, etc.). This is accomplished by providing an affinity matrix bearing nucleic acid affinity ligands complementary to the gene products (e.g., mRNAs or nucleic acids derived therefrom) or to subsequences thereof. Hybridization of the nucleic acid sample to the affinity matrix will result in duplex formation between the affinity ligands and their target nucleic acids. Upon elution of the sample from the affinity matrix, the matrix will retain the duplexes nucleic acids leaving a sample depleted of the overexpressed target nucleic acids.

The affinity matrix can also be used to identify unknown mRNAs or cDNAs in a sample. Where the affinity matrix contains nucleic acids complementary to every known gene (e.g., in a cDNA library, DNA reverse transcribed from an mRNA, mRNA used directly or amplified, or polymerized from a DNA template) in a sample, capture of the known nucleic acids by the affinity matrix leaves a sample enriched for those nucleic acid sequences that are unknown. In effect, the affinity matrix is used to perform a subtractive hybridization to isolate unknown nucleic acid sequences. The remaining "unknown" sequences can then be purified and sequenced according to standard methods.

The affinity matrix can also be used to capture (isolate) and thereby purify unknown nucleic acid sequences. For example, an affinity matrix can be prepared that

contains nucleic acid (affinity ligands) that are complementary to sequences not previously identified, or not previously known to be expressed in a particular nucleic acid sample. The sample is then hybridized to the affinity matrix and those sequences that are retained on the affinity matrix are "unknown" nucleic acids. The retained nucleic acids can be eluted from the matrix (e.g. at increased temperature, increased destabilizing agent concentration, or decreased salt) and the nucleic acids can then be sequenced according to standard methods.

Similarly, the affinity matrix can be used to efficiently capture (isolate) a number of known nucleic acid sequences. Again, the matrix is prepared bearing nucleic acids complementary to those nucleic acids it is desired to isolate. The sample is contacted to the matrix under conditions where the complementary nucleic acid sequences hybridize to the affinity ligands in the matrix. The non-hybridized material is washed off the matrix leaving the desired sequences bound. The hybrid duplexes are then denatured providing a pool of the isolated nucleic acids. The different nucleic acids in the pool can be subsequently separated according to standard methods (e.g. gel electrophoresis).

As indicated above the affinity matrices can be used to selectively remove nucleic acids from virtually any sample containing nucleic acids (e.g., in a cDNA library, DNA reverse transcribed from an mRNA, mRNA used directly or amplified, or polymerized from a DNA template, and so forth). The nucleic acids adhering to the column can be removed by washing with a low salt concentration buffer, a buffer containing a destabilizing agent such as formamide, or by elevating the column temperature.

In one particularly preferred embodiment, the affinity matrix can be used in a method to enrich a sample for unknown RNA sequences (e.g. expressed sequence tags (ESTs)). The method involves first providing an affinity matrix bearing a library of oligonucleotide probes specific to known RNA (e.g., EST) sequences. Then, RNA from undifferentiated and/or unactivated cells and RNA from differentiated or activated or pathological (e.g., transformed) or otherwise having a different metabolic state are separately hybridized against the affinity matrices to provide two pools of RNAs lacking the known RNA sequences.

In a preferred embodiment, the affinity matrix is packed into a columnar casing. The sample is then applied to the affinity matrix (e.g. injected onto a column or applied to a column by a pump such as a sampling pump driven by an autosampler). The affinity matrix (e.g. affinity column) bearing the sample is subjected to conditions under which the nucleic acid probes comprising the affinity matrix hybridize specifically with complementary target nucleic acids. Such conditions are accomplished by maintaining appropriate pH, salt and temperature conditions to facilitate hybridization as discussed above.

For a number of applications, it may be desirable to extract and separate messenger RNA from cells, cellular debris, and other contaminants. As such, the device of the present invention may, in some cases, include an mRNA purification chamber or channel. In general, such purification takes advantage of the poly-A tails on mRNA. In particular and as noted above, poly- T oligonucleotides may be immobilized within a chamber or channel of the device to serve as affinity ligands for mRNA. Poly-T oligonucleotides may be immobilized upon a solid support incorporated within the chamber or channel, or alternatively, may be immobilized upon the surface(s) of the chamber or channel itself. Immobilization of oligonucleotides on the surface of the chambers or channels may be carried out by methods described herein including, e.g., oxidation and silanation of the surface followed by standard DMT synthesis of the oligonucleotides.

In operation, the lysed sample is introduced to a high salt solution to increase the ionic strength for hybridization, whereupon the mRNA will hybridize to the immobilized poly-T. The mRNA bound to the immobilized poly-T oligonucleotides is then washed free in a low ionic strength buffer. The poy-T oligonucleotides may be immobiliized upon poroussurfaces, e.g., porous silicon, zeolites silica xerogels, scintered particles, or other solid supports.

Hybridization

Following sample preparation, the sample can be subjected to one or more different analysis operations. A variety of analysis operations may generally be performed, including size based analysis using, e.g., microcapillary electrophoresis, and/or sequence based analysis using, e.g., hybridization to an oligonucleotide array.

In the latter case, the nucleic acid sample may be probed using an array of oligonucleotide probes. Oligonucleotide arrays generally include a substrate having a large number of positionally distinct oligonucleotide probes attached to the substrate. These arrays may be produced using mechanical or light directed synthesis methods which incorporate a combination of photolithographic methods and solid phase oligonucleotide synthesis methods.

Light directed synthesis of oligonucleotide arrays

The basic strategy for light directed synthesis of oligonucleotide arrays is as follows. The surface of a solid support, modified with photosensitive protecting groups is illuminated through a photolithographic mask, yielding reactive hydroxyl groups in the illuminated regions. A selected nucleotide, typically in the form of a 3'-O-phosphoramidite-activated deoxynucleoside (protected at the 5' hydroxyl with a photosensitive protecting group), is then presented to the surface and coupling occurs at the sites that were exposed to light. Following capping and oxidation, the substrate is rinsed and the surface is illuminated through a second mask, to expose additional hydroxyl groups for coupling. A second selected nucleotide (e.g., 5'-protected, 3'-O-phosphoramidite-activated deoxynucleoside) is presented to the surface. The selective deprotection and coupling cycles are repeated until the desired set of products is obtained. Since photolithography is used, the process can be readily miniaturized to generate high density arrays of oligonucleotide probes. Furthermore, the sequence of the oligonucleotides at each site is known. See, Pease, et al. Mechanical synthesis methods are similar to the light directed methods except involving mechanical direction of fluids for deprotection and addition in the synthesis steps.

For some embodiments, oligonucleotide arrays may be prepared having all possible probes of a given length. The hybridization pattern of the target sequence on the array may be used to reconstruct the target DNA sequence. Hybridization analysis of large numbers of probes can be used to sequence long stretches of DNA or provide an oligonucleotide array which is specific and complementary to a particular nucleic acid sequence. For example, in particularly preferred aspects, the oligonucleotide array will contain oligonucleotide probes which are complementary

to specific target sequences, and individual or multiple mutations of these. Such arrays are particularly useful in the diagnosis of specific disorders which are characterized by the presence of a particular nucleic acid sequence.

Following sample collection and nucleic acid extraction, the nucleic acid portion of the sample is typically subjected to one or more preparative reactions. These preparative reactions include in vitro transcription, labeling, fragmentation, amplification and other reactions. Nucleic acid amplification increases the number of copies of the target nucleic acid sequence of interest. A variety of amplification methods are suitable for use in the methods and device of the present invention, including for example, the polymerase chain reaction method or (PCR), the ligase chain reaction (LCR), self sustained sequence replication (3SR), and nucleic acid based sequence amplification (NASBA).

The latter two amplification methods involve isothermal reactions based on isothermal transcription, which produce both single stranded RNA (ssRNA) and double stranded DNA (dsDNA) as the amplification products in a ratio of approximately 30 or 100 to 1, respectively. As a result, where these latter methods are employed, sequence analysis may be carried out using either type of substrate, i.e., complementary to either DNA or RNA.

Frequently, it is desirable to amplify the nucleic acid sample prior to hybridization. One of skill in the art will appreciate that whatever amplification method is used, if a quantitative result is desired, care must be taken to use a method that maintains or controls for the relative frequencies of the amplified nucleic acids.

PCR

Methods of "quantitative" amplification are well known to those of skill in the art. For example, quantitative PCR involves simultaneously co-amplifying a known quantity of a control sequence using the same primers. This provides an internal standard that may be used to calibrate the PCR reaction. The high density array may then include probes specific to the internal standard for quantification of the amplified nucleic acid.

Thus, in one embodiment, this invention provides for a method of optimizing a probe set for detection of a particular gene. Generally, this method involves providing a high density array containing a multiplicity of probes of one or more particular length(s) that are complementary to subsequences of the mRNA transcribed by the target gene. In one embodiment the high density array may contain every probe of a particular length that is complementary to a particular mRNA. The probes of the high density array are then hybridized with their target nucleic acid alone and then hybridized with a high complexity, high concentration nucleic acid sample that does not contain the targets complementary to the probes. Thus, for example, where the target nucleic acid is an RNA, the probes are first hybridized with their target nucleic acid alone and then hybridized with RNA made from a cDNA library (e.g., reverse transcribed polyA.sup.+ mRNA) where the sense of the hybridized RNA is opposite that of the target nucleic acid (to insure that the high complexity sample does not contain targets for the probes). Those probes that show a strong hybridization signal with their target and little or no cross-hybridization with the high complexity sample are preferred probes for use in the high density arrays of this invention.

PCR amplification generally involves the use of one strand of the target nucleic acid sequence as a template for producing a large number of complements to that sequence. Generally, two primer sequences complementary to different ends of a segment of the complementary strands of the target sequence hybridize with their respective strands of the target sequence, and in the presence of polymerase enzymes and nucleoside triphosphates, the primers are extended along the target sequence. The extensions are melted from the target sequence and the process is repeated, this time with the additional copies of the target sequence synthesized in the preceding steps. PCR amplification typically involves repeated cycles of denaturation, hybridization and extension reactions to produce sufficient amounts of the target nucleic acid. The first step of each cycle of the PCR involves the separation of the nucleic acid duplex formed by the primer extension. Once the strands are separated, the next step in PCR involves hybridizing the separated strands with primers that flank the target sequence. The primers are then extended to form complementary copies of the target strands. For successful PCR amplification, the primers are designed so that the position at which each primer hybridizes along a duplex sequence is such that an extension product synthesized from one primer, when separated from the template (complement), serves as a

template for the extension of the other primer. The cycle of denaturation, hybridization, and extension is repeated as many times as necessary to obtain the desired amount of amplified nucleic acid.

In PCR methods, strand separation is normally achieved by heating the reaction to a sufficiently high temperature for a sufficient time to cause the denaturation of the duplex but not to cause an irreversible denaturation of the polymerase. Typical heat denaturation involves temperatures ranging from about 80.degree. C. to 105.degree. C. for times ranging from seconds to minutes. Strand separation, however, can be accomplished by any suitable denaturing method including physical, chemical, or enzymatic means. Strand separation may be induced by a helicase, for example, or an enzyme capable of exhibiting helicase activity.

In addition to PCR and IVT reactions, the methods and devices of the present invention are also applicable to a number of other reaction types, e.g., reverse transcription, nick translation, and the like.

Labelling before hybridization

The nucleic acids in a sample will generally be labeled to facilitate detection in subsequent steps. Labeling may be carried out during the amplification, in vitro transcription or nick translation processes. In particular, amplification, in vitro transcription or nick translation may incorporate a label into the amplified or transcribed sequence, either through the use of labeled primers or the incorporation of labeled dNTPs into the amplified sequence.

Hybridization between the sample nucleic acid and the oligonucleotide probes upon the array is then detected, using, e.g., epifluorescence confocal microscopy. Typically, sample is mixed during hybridization to enhance hybridization of nucleic acids in the sample to nucleoc acid probes on the array.

Labelling after hybridization

In some cases, hybridized oligonucleotides may be labeled following hybridization. For example, where biotin labeled dNTPs are used in, e.g., amplification or

transcription, streptavidin linked reporter groups may be used to label hybridized complexes. Such operations are readily integratable into the systems of the present invention. Alternatively, the nucleic acids in the sample may be labeled following amplification. Post amplification labeling typically involves the covalent attachment of a particular detectable group upon the amplified sequences. Suitable labels or detectable groups include a variety of fluorescent or radioactive labeling groups well known in the art. These labels may also be coupled to the sequences using methods that are well known in the art.

Methods for detection depend upon the label selected. A fluorescent label is preferred because of its extreme sensitivity and simplicity. Standard labeling procedures are used to determine the positions where interactions between a sequence and a reagent take place. For example, if a target sequence is labeled and exposed to a matrix of different probes, only those locations where probes do interact with the target will exhibit any signal. Alternatively, other methods may be used to scan the matrix to determine where interaction takes place. Of course, the spectrum of interactions may be determined in a temporal manner by repeated scans of interactions which occur at each of a multiplicity of conditions. However, instead of testing each individual interaction separately, a multiplicity of sequence interactions may be simultaneously determined on a matrix.

Means of detecting labeled target (sample) nucleic acids hybridized to the probes of the high density array are known to those of skill in the art. Thus, for example, where a colorimetric label is used, simple visualization of the label is sufficient. Where a radioactive labeled probe is used, detection of the radiation (e.g with photographic film or a solid state detector) is sufficient.

In a preferred embodiment, however, the target nucleic acids are labeled with a fluorescent label and the localization of the label on the probe array is accomplished with fluorescent microscopy. The hybridized array is excited with a light source at the excitation wavelength of the particular fluorescent label and the resulting fluorescence at the emission wavelength is detected. In a particularly preferred embodiment, the excitation light source is a laser appropriate for the excitation of the fluorescent label.

The target polynucleotide may be labeled by any of a number of convenient detectable markers. A fluorescent label is preferred because it provides a very strong signal with low background. It is also optically detectable at high resolution and sensitivity through a quick scanning procedure. Other potential labeling moieties include, radioisotopes, chemiluminescent compounds, labeled binding proteins, heavy metal atoms, spectroscopic markers, magnetic labels, and linked enzymes. Another method for labeling may bypass any label of the target sequence. The target may be exposed to the probes, and a double strand hybrid is formed at those positions only. Addition of a double strand specific reagent will detect where hybridization takes place. An intercalative dye such as ethidium bromide may be used as long as the probes themselves do not fold back on themselves to a significant extent forming hairpin loops. However, the length of the hairpin loops in short oligonucleotide probes would typically be insufficient to form a stable duplex.

Suitable chromogens will include molecules and compounds which absorb light in a distinctive range of wavelengths so that a color may be observed, or emit light when irradiated with radiation of a particular wave length or wave length range, e.g., fluorescers. Biliproteins, e.g., phycoerythrin, may also serve as labels.

A wide variety of suitable dyes are available, being primarily chosen to provide an intense color with minimal absorption by their surroundings. Illustrative dye types include quinoline dyes, triarylmethane dyes, acridine dyes, alizarine dyes, phthaleins, insect dyes, azo dyes, anthraquinoid dyes, cyanine dyes, phenazathionium dyes, and phenazoxonium dyes.

A wide variety of fluorescers may be employed either by themselves or in conjunction with quencher molecules. Fluorescers of interest fall into a variety of categories having certain primary functionalities. These primary functionalities include 1- and 2-aminonaphthalene, p,p'-diaminostilbenes, pyrenes, quaternary phenanthridine salts, 9-aminoacridines, p,p'-diaminobenzophenone imines, anthracenes, oxacarbocyanine, merocyanine, 3-aminoequilenin, perylene, bis-benzoxazole, bis-p-oxazolyl benzene, 1,2-benzophenazin, retinol, bis-3-aminopyridinium salts, hellebrigenin, tetracycline, sterophenol, benzimidzaolylphenylamine, 2-oxo-3-chromen, indole, xanthen, 7-hydroxycoumarin, phenoxazine, salicylate, strophanthidin, porphyrins, triarylmethanes and flavin.

Individual fluorescent compounds which have functionalities for linking or which can be modified to incorporate such functionalities include, e.g., dansyl chloride; fluoresceins such as 3,6-dihydroxy-9-phenylxanthhydrol; rhodamineisothiocyanate; N-phenyl 1-amino-8-sulfonatonaphthalene; N-phenyl 2-amino-6-sulfonatonaphthalene; 4-acetamido-4-isothiocyanato-stilbene-2,2'-disulfonic acid; pyrene-3-sulfonic acid; 2-toluidinonaphthalene-6-sulfonate; N-phenyl, N-methyl 2-aminoaphthalene-6-sulfonate; ethidium bromide; stebrine; auromine-0,2-(9'-anthroyl)palmitate; dansyl phosphatidylethanolamine; N,N'-dioctadecyl oxacarbocyanine; N,N'-dihexyl oxacarbocyanine; merocyanine, 4-(3'pyrenyl)butyrate; d-3-aminodesoxy-equilenin; 12-(9'-anthroyl)stearate; 2-methylanthracene; 9-vinylanthracene; 2,2'-(vinylene-p-phenylene)bisbenzoxazole; p-bis›2-(4-methyl-5-phenyl-oxazolyl)!benzene; 6-dimethylamino-1,2-benzophenazin; retinol; bis(3'-aminopyridinium) 1,10-decandiyl diiodide; sulfonaphthylhydrazone of hellibrienin; chlorotetracycline; N-(7-dimethylamino-4-methyl-2-oxo-3-chromenyl)maleimide; N-›p-(2-benzimidazolyl)-phenyl!maleimide; N-(4-fluoranthyl)maleimide; bis(homovanillic acid); resazarin; 4-chloro-7-nitro-2,1,3-benzooxadiazole; merocyanine 540; resorufin; rose bengal; and 2,4-diphenyl-3(2H)-furanone.

Desirably, fluorescers should absorb light above about 300 nm, preferably about 350 nm, and more preferably above about 400 nm, usually emitting at wavelengths greater than about 10 nm higher than the wavelength of the light absorbed. It should be noted that the absorption and emission characteristics of the bound dye may differ from the unbound dye. Therefore, when referring to the various wavelength ranges and characteristics of the dyes, it is intended to indicate the dyes as employed and not the dye which is unconjugated and characterized in an arbitrary solvent.

Fluorescers are generally preferred because by irradiating a fluorescer with light, one can obtain a plurality of emissions. Thus, a single label can provide for a plurality of measurable events.

Detectable signal may also be provided by chemiluminescent and bioluminescent sources. Chemiluminescent sources include a compound which becomes electronically excited by a chemical reaction and may then emit light which serves

as the detectible signal or donates energy to a fluorescent acceptor. A diverse number of families of compounds have been found to provide chemiluminescence under a variety of conditions. One family of compounds is 2,3-dihydro-1,-4-phthalazinedione. The most popular compound is luminol, which is the 5-amino compound. Other members of the family include the 5-amino-6,7,8-trimethoxy- and the dimethylamino›ca!benz analog. These compounds can be made to luminesce with alkaline hydrogen peroxide or calcium hypochlorite and base. Another family of compounds is the 2,4,5-triphenylimidazoles, with lophine as the common name for the parent product. Chemiluminescent analogs include para-dimethylamino and -methoxy substituents. Chemiluminescence may also be obtained with oxalates, usually oxalyl active esters, e.g., p-nitrophenyl and a peroxide, e.g., hydrogen peroxide, under basic conditions. Alternatively, luciferins may be used in conjunction with luciferase or lucigenins to provide bioluminescence.

Spin labels are provided by reporter molecules with an unpaired electron spin which can be detected by electron spin resonance (ESR) spectroscopy. Exemplary spin labels include organic free radicals, transitional metal complexes, particularly vanadium, copper, iron, and manganese, and the like. Exemplary spin labels include nitroxide free radicals.

Fragmentation

In addition, amplified sequences may be subjected to other post amplification treatments. For example, in some cases, it may be desirable to fragment the sequence prior to hybridization with an oligonucleotide array, in order to provide segments which are more readily accessible to the probes, which avoid looping and/or hybridization to multiple probes. Fragmentation of the nucleic acids may generally be carried out by physical, chemical or enzymatic methods that are known in the art.

Sample Analysis

Following the various sample preparation operations, the sample will generally be subjected to one or more analysis operations. Particularly preferred analysis operations include, e.g., sequence based analyses using an oligonucleotide array

and/or size based analyses using, e.g., microcapillary array electrophoresis.

Capillary Electrophoresis

In some embodiments, it may be desirable to provide an additional, or alternative means for analyzing the nucleic acids from the sample

Microcapillary array electrophoresis generally involves the use of a thin capillary or channel which may or may not be filled with a particular separation medium. Electrophoresis of a sample through the capillary provides a size based separation profile for the sample. Microcapillary array electrophoresis generally provides a rapid method for size based sequencing, PCR product analysis and restriction fragment sizing. The high surface to volume ratio of these capillaries allows for the application of higher electric fields across the capillary without substantial thermal variation across the capillary, consequently allowing for more rapid separations. Furthermore, when combined with confocal imaging methods, these methods provide sensitivity in the range of attomoles, which is comparable to the sensitivity of radioactive sequencing methods.

In many capillary electrophoresis methods, the capillaries, e.g., fused silica capillaries or channels etched, machined or molded into planar substrates, are filled with an appropriate separation/sieving matrix. Typically, a variety of sieving matrices are known in the art may be used in the microcapillary arrays. Examples of such matrices include, e.g., hydroxyethyl cellulose, polyacrylamide, agarose and the like. Gel matrices may be introduced and polymerized within the capillary channel. However, in some cases, this may result in entrapment of bubbles within the channels which can interfere with sample separations. Accordingly, it is often desirable to place a preformed separation matrix within the capillary channel(s), prior to mating the planar elements of the capillary portion. Fixing the two parts, e.g., through sonic welding, permanently fixes the matrix within the channel. Polymerization outside of the channels helps to ensure that no bubbles are formed. Further, the pressure of the welding process helps to ensure a void-free system.

In addition to its use in nucleic acid "fingerprinting" and other sized based analyses, the capillary arrays may also be used in sequencing applications. In particular, gel

based sequencing techniques may be readily adapted for capillary array electrophoresis.

Expression products

In addition to detection of mRNA or as the sole detection method expression products from the genes discussed above may be detected as indications of the biological condition of the tissue. Expression products may be detected in either the tissue sample as such, or in a body fluid sample, such as blood, serum, plasma, faeces, mucus, sputum, cerebrospinal fluid, and/or urine of the individual.

The expression products, peptides and proteins, may be detected by any suitable technique known to the person skilled in the art.

In a preferred embodiment the expression products are detected by means of specific antibodies directed to the various expression products, such as immunofluorescent and/or immunohistochemical staining of the tissue.

Immunohistochemical localization of expressed proteins may be carried out by immunostaining of tissue sections from the single tumors to determine which cells expressed the protein encoded by the transcript in question. The transcript levels were used to select a group of proteins supposed to show variation from sample to sample, making possible a rough correlation between level of protein detected and intensity of the transcript on the microarray.

For example sections were cut from paraffin-embedded tissue blocks, mounted, and deparaffinized by incubation at 80 C° for 10 min, followed by immersion in heated oil at 60 C for 10 min (Estisol 312, Estichem A/S, Denmark) and rehydration.. Antigen retrieval is achieved in TEG (TrisEDTA-Glycerol) buffer using microwaves at 900 W. The tissue sections cooled in the buffer for 15 min before a brief rinse in tap water. Endogenous peroxidase activity is blocked by incubating the sections with 1% H202 for 20 min, followed by three rinses in tap water, 1 min each. The sections are then soaked in PBS buffer for 2 min. The next steps are modified from the descriptions given by Oncogene Science Inc., in the Mouse Immunohistochemistry Detection System, XHCO1 (UniTect, Uniondale, NY, USA). Briefly, the tissue sections are

incubated overnight at 4 C with primary antibody (against beta-2 microglobulin (Dako), cytokeratin 8, cystatin-C (both from Europa, US), junB, CD59, E-cadherin, apo-E, cathepsin E, vimentin, IGFII (all from Santa Cruz), followed by three rinses in PBS buffer for 5 min each. Afterwards, the sections are incubated with biotinylated secondary antibody for 30 min, rinsed three times with PBS buffer and subsequently incubated with ABC (avidin-biotinlylated horseradish peroxidase complex) for 30 min, followed by three rinses in PBS buffer.

Staining is performed by incubation with AEC (3-amino-ethylcarbazole) for 10 min. The tissue sections are counter stained with Mayers hematoxylin, washed in tap water for 5 min. and mounted with glycerol-gelatin. Positive and negative controls may be included in each staining round with all antibodies.

In yet another embodiment the expression products may be detected by means of conventional enzyme assays, such as ELISA methods.

Furthermore, the expression products may be detected by means of peptide/protein chips capable of specifically binding the peptides and/or proteins assessed. Thereby an expression pattern may be obtained.

Assay

Thus, in a further aspect the invention relates to an assay for determining an expression pattern of a colon and/or rectum cell, comprising at least a first marker and/or a second marker, wherein the first marker is capable of detecting a gene from a first gene group as defined above, and the second marker is capable of detecting a gene from a second gene group as defined above.

In a preferred embodiment the assay comprises at least two markers for each gene group.

correlating the first expression level and the second expression level to a standard level of the assessed genes to determine the presence or absence of a biological condition in the animal tissue.

The marker (s) are preferably specifically detecting a gene as identified herein, in particular the genes of the tables in the examples and as discussed above.

As discussed above the marker may be any nucleotide probe, such as a DNA, RNA, PNA, or LNA probe capable of hybridising to mRNA indicative of the expression level. The hybridisation conditions are preferably as described below for probes.

In another embodiment the marker is an antibody capable of specifically binding the expression product in question.

Detection

Patterns can be compared manually by a person or by a computer or other machine. An algorithm can be used to detect similarities and differences. The algorithm may score and compare, for example, the genes which are expressed and the genes which are not expressed. Alternatively, the algorithm may look for changes in intensity of expression of a particular gene and score changes in intensity between two samples. Similarities may be determined on the basis of genes which are expressed in both samples and genes which are not expressed in both samples or on the basis of genes whose intensity of expression are numerically similar.

Generally, the detection operation will be performed using a reader device external to the diagnostic device. However, it may be desirable in some cases, to incorporate the data gathering operation into the diagnostic device itself.

The detection apparatus may be a fluorescence detector, or a spectroscopic detector, or another detector.

Although hybridization is one type of specific interaction which is clearly useful for use in this mapping embodiment, antibody reagents may also be very useful.

Data Gathering and Analysis

Gathering data from the various analysis operations, e.g., oligonucleotide and/or microcapillary arrays, will typically be carried out using methods known in the art.

For example, the arrays may be scanned using lasers to excite fluorescently labeled targets that have hybridized to regions of probe arrays mentioned above, which can then be imaged using charged coupled devices ("CCDs") for a wide field scanning of the array. Alternatively, another particularly useful method for gathering data from the arrays is through the use of laser confocal microscopy which combines the ease and speed of a readily automated process with high resolution detection.

Following the data gathering operation, the data will typically be reported to a data analysis operation. To facilitate the sample analysis operation, the data obtained by the reader from the device will typically be analyzed using a digital computer. Typically, the computer will be appropriately programmed for receipt and storage of the data from the device, as well as for analysis and reporting of the data gathered, i.e., interpreting fluorescence data to determine the sequence of hybridizing probes, normalization of background and single base mismatch hybridizations, ordering of sequence data in SBH applications, and the like.

It is an object of the present invention to provide a biological sample which may be classified or characterized by analyzing the pattern of specific interactions mentioned above. This may be applicable to a cell or tissue type, to the messenger RNA population expressed by a cell to the genetic content of a cell, or to virtually any sample which can be classified and/or identified by its combination of specific molecular properties.

Pharmaceutical composition

The invention also relates to a pharmaceutical composition for treating the bioligical condition, such as colorectal tumors.

In one embodiment the pharmaceutical composition comprises one or more of the peptides being expression products as defined above. In a preferred embodiment, the peptides are bound to carriers. The peptides may suitably be coupled to a polymer carrier, for example a protein carrier, such as BSA. Such formulations are well-known to the person skilled in the art.

The peptides may be suppressor peptides normally lost or decreased in tumor tissue administered in order to stabilise tumors towards a less malignant stage. In an-

other embodiment the peptides are onco-peptides capable of eliciting an immune response towards the tumor cells.

In another embodiment the pharmaceutical composition comprises genetic material, either genetic material for substitution therapy, or for suppressing therapy as discussed below.

In a third embodiment the pharmaceutical composition comprises at least one antibody produced as described above.

In the present context the term pharmaceutical composition is used synonymously with the term medicament. The medicament of the invention comprises an effective amount of one or more of the compounds as defined above, or a composition as defined above in combination with pharmaceutically acceptable additives. Such medicament may suitably be formulated for oral, percutaneous, intramuscular, intravenous, intracranial, intrathecal, intracerebroventricular, intranasal or pulmonal administration. For most indications a localised or substantially localised application is preferred.

Strategies in formulation development of medicaments and compositions based on the compounds of the present invention generally correspond to formulation strategies for any other protein-based drug product. Potential problems and the guidance required to overcome these problems are dealt with in several textbooks, e.g. "Therapeutic Peptides and Protein Formulation. Processing and Delivery Systems", Ed. A.K. Banga, Technomic Publishing AG, Basel, 1995.

Injectables are usually prepared either as liquid solutions or suspensions, solid forms suitable for solution in, or suspension in, liquid prior to injection. The preparation may also be emulsified. The active ingredient is often mixed with excipients which are pharmaceutically acceptable and compatible with the active ingredient. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol or the like, and combinations thereof. In addition, if desired, the preparation may contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents, or which enhance the effectiveness or transportation of the preparation.

Formulations of the compounds of the invention can be prepared by techniques known to the person skilled in the art. The formulations may contain pharmaceutically acceptable carriers and excipients including microspheres, liposomes, microcapsules, nanoparticles or the like.

The preparation may suitably be administered by injection, optionally at the site, where the active ingredient is to exert its effect. Additional formulations which are suitable for other modes of administration include suppositories, and, in some cases, oral formulations. For suppositories, traditional binders and carriers include polyalkylene glycols or triglycerides. Such suppositories may be formed from mixtures containing the active ingredient(s) in the range of from 0.5% to 10%, preferably 1-2%. Oral formulations include such normally employed excipients as, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, and the like. These compositions take the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations or powders and generally contain 10-95% of the active ingredient(s), preferably 25-70%.

The preparations are administered in a manner compatible with the dosage formulation, and in such amount as will be therapeutically effective. The quantity to be administered depends on the subject to be treated, including, e.g. the weight and age of the subject, the disease to be treated and the stage of disease. Suitable dosage ranges are of the order of several hundred μg active ingredient per administration with a preferred range of from about 0.1 μg to 1000 μg, such as in the range of from about 1 μg to 300 μg, and especially in the range of from about 10 μg to 50 μg. Administration may be performed once or may be followed by subsequent administrations. The dosage will also depend on the route of administration and will vary with the age and weight of the subject to be treated. A preferred dosis would be in the interval 30 mg to 70 mg per 70 kg body weight.

Some of the compounds of the present invention are sufficiently active, but for some of the others, the effect will be enhanced if the preparation further comprises pharmaceutically acceptable additives and/or carriers. Such additives and carriers will be known in the art. In some cases, it will be advantageous to include a compound,

which promote delivery of the active substance to its target.

In many instances, it will be necessary to administrate the formulation multiple times. Administration may be a continuous infusion, such as intraventricular infusion or administration in more doses such as more times a day, daily, more times a week, weekly, etc.

Vaccines

In a further embodiment the present invention relates to a vaccine for the prophylaxis or treatment of a biological condition comprising at least one expression product from at least one gene said gene being expressed as defined above.

The term vaccines is used with its normal meaning, i.e preparations of immunogenic material for administration to induce in the recipient an immunity to infection or intoxication by a given infecting agent. Vaccines may be administered by intravenous injection or through oral, nasal and/or mucosal administration. Vaccines may be either simple vaccines prepared from one species of expression products, such as proteins or peptides, or a variety of expression products, or they may be mixed vaccines containing two or more simple vaccines. They are prepared in such a manner as not to destroy the immunogenic material, although the methods of preparation vary, depending on the vaccine.

The enhanced immune response achieved according to the invention can be attributable to e.g. an enhanced increase in the level of immunoglobulins or in the level of T-cells including cytotoxic T-cells will result in immunisation of at least 50% of individuals exposed to said immunogenic composition or vaccine, such as at least 55%, for example at least 60%, such as at least 65%, for example at least 70%, for example at least 75%, such as at least 80%, for example at least 85%, such as at least 90%, for example at least 92%, such as at least 94%, for example at least 96%, such as at least 97%, for example at least 98%, such as at least 98.5%, for example at least 99%, for example at least 99.5% of the individuals exposed to said immunogenic composition or vaccine are immunised.

Compositions according to the invention may also comprise any carrier and/or adjuvant known in the art including functional equivalents thereof. Functionally equivalent carriers are capable of presenting the same immunogenic determinant in essentially the same steric conformation when used under similar conditions. Functionally equivalent adjuvants are capable of providing similar increases in the efficacy of the composition when used under similar conditions.

Therapy

The invention further relates to a method of treating individuals suffering from the biological condition in question, in particular for treating a colorectal tumor.

In one embodiment the invention relates to a method of substitution therapy, ie. administration of genetic material generally expressed in normal cells, but lost or decreased in biological condition cells(tumor suppressors). Thus, the invention relates to a method for reducing cell tumorigenicity of a cell, said method comprising

obtaining at least one gene selected from genes being expressed in an amount two-fold higher in normal cells than the amount expressed in said tumor cell(tumor suppressors),

introducing said at least one gene into the tumor cell in a manner allowing expression of said gene(s).

The at least one gene is preferably selected individually from genes comprising a sequence as identified below

RC_H04768_at		*chrom 15 no homology*
RC_Z39652_at		*Y14593 APM-1 gene adipocyte-specific secretory protein; chrom 1q21.3-q23*
RC_H30270_at		**chrom 18 PAAAA in colon & bladder *no homology***
RC_T47089_s_at		tenascin-X; tenascin-X precursor; unidentified protein
RC_W31906_at		secretagogin; dJ501N12.8 (putative protein) chrom 6
RC_AA279803_at		*chrom 2 no homology*
RC_R01646_at		chrom ***13q32.1-33.3 ; AL159152 ; homology to mouse Pcbp1 - poly(rC)-binding***

		protein 1
AA319615_at		secretory carrier membrane protein; secretory carrier membrane protein 2; chrom 15

and from

"Human chromogranin A ""mRNA,"" complete cds"	**J03915**
Human adipsin/complement factor D "mRNA," complete cds	**M84526**
Homo sapiens MLC-1V/Sb isoform gene	**M24248**
Human aminopeptidase N/CD13 mRNA encoding aminopeptidase "N," complete cds	**M22324**
H.sapiens MT-1I mRNA	**X76717**
H.sapiens GCAP-II gene	**Z70295**
Human somatostatin I gene and flanks	**J00306**
Human YMP "mRNA," complete cds	U52101
H.sapiens mRNA for beta subunit of epithelial amiloride-sensitive sodium channel	X87159
Human K12 protein precursor "mRNA," complete cds	U77643
Human sulfate transporter (DTD) "mRNA," complete cds	U14528
Human transcription factor hGATA-6 "mRNA," complete cds.	U66075
H.sapiens SCAD "gene," exon 1 and joining features	Z80345
Human S-lac lectin L-14-II (LGALS2) gene	M87860
Human mRNA for protein tyrosine phosphatase	D15049
H.sapiens mRNA for tetranectin	X64559
Human 11kd protein "mRNA," complete cds	U28249
Human anti-mullerian hormone type II receptor precursor "gene," complete cds	U29700
Human heparin binding protein (HBp17) "mRNA," complete cds	M60047
Human ADP-ribosylation factor (hARF6) "mRNA," complete cds	M57763
beta -ADD=adducin beta subunit 63 kda isoform/membrane skeleton protein, beta -ADD=adducin beta subunit 63 kda isoform/membrane skeleton protein {alternatively spliced, exon 10 to 13 region} [human, Genomic, 1851 nt, segment 3 of 3].	S81083
Zinc Finger Protein Znf155	HG4243-HT4513
Human glucagon "mRNA," complete cds	J04040
H.sapiens mRNA for hair "keratin," hHb5	X99140
Human tubulin-folding cofactor E "mRNA," complete cds	U61232
Human integrin alpha-3 chain "mRNA," complete cds	M59911
Human NACP gene	U46901
H.sapiens mRNA for flavin-containing monooxygenase 5 (FMO5)	Z47553
Human mRNA for ATF-a transcription factor	X52943
H.sapiens intestinal VIP receptor related protein mRNA	X77777

In a preferred embodiment at least two different genes are introduced into the tumor cell.

In another aspect the invention relates to a therapy whereby genes generally correlated to disease are inhibited by one or more of the following methods:

A method for reducing cell tumorigenicity of a cell, said method comprising

obtaining at least one nucleotide probe capable of hybridising with at least one gene of a tumor cell, said at least one gene being selected from genes being expressed in an amount at least one-fold lower in normal cells than the amount expressed in said tumor cell, and

introducing said at least one nucleotide probe into the tumor cell in a manner allowing the probe to hybridise to the at least one gene, thereby inhibiting expression of said at least one gene. This method is preferably based on anti-sense technology, whereby the hybridisation of said probe to the gene leads to a down-regulation of said gene.

The down-regulation may of course also be based on a probe capable of hybridising to regulatory components of the genes in question, such as promoters.

The probes are preferably selected from probes capable of hybridising to a nucleotide sequence comprising a sequence as identified below

RC_AA609013_s_at	APPPP	microsomal dipeptidase (also on 6.8k); chrom 16
RC_AA232508_at	APPPP	CGI-89 protein; unnamed protein product; hypothetical protein
RC_AA428964_at	APPPP	serine protease-like protease; serine protease homolog=NES1; normal epithelial cell-specific 1
RC_T52813_s_at	APPPP	dJ28O10.2 (G0S2 (PUTATIVE LYMPHOCYTE G0/G1 SWITCH PROTEIN 2; chrom 1
RC_AA075642_at	APPPP	gp-340 variant protein; DMBT1/8kb.2 protein
RC_AA007218_at	APPP	*chrom 13 no homology*

	P	
RC_N33920_at	APPP P	ubiquitin-like protein FAT10; diubiquitin; dJ271M21.6 (Diubiquitin); chrom 6
RC_N71781_at	APPP P	KIAA1199 protein, chrom 15
RC_R67275_s_at	APPP P	alpha-1 (type XI) collagen precursor; collagen, type XI, alpha 1; collagen type XI alpha-1 isoform A; chrom 1
RC_W80763_at	APPP P	hypothetical protein; chrom 17
RC_AA443793_at	APPP P	*chrom 7p22 AC006028 BAC clone*
RC_AA034499_s_at	APPP P	ZNF198 protein; zinc finger protein; FIM protein; Cys-rich protein; zinc finger protein 198; chrom 13
RC_AA035482_at	APPP P	*chrom 5; AK022505 clone; CalcineurinB (weakly similar)*
RC_AA024482_at	APPP P	hypothetical protein; unnamed protein product; chrom 17
RC_H93021_at	APPP P	*chrom 2 ; XM_004890 peptidylprolyl isomerase A (cyclophilin A)*
RC_AA427737_at	APPP P	*no homology*
RC_AA417078_at	APPP P	*chrom 7q31; AF017104 clone*
M29873_s_at	APPP P	**cytochrome P450-IIB (hIIB3) ; 19q13.1-q13.2**
RC_H27498_f_at	AAPP P	
RC_T92363_s_at	AAPP P	
RC_N89910_at	AAAP P	
RC_W60516_at	AAAP P	
RC_AA219699_at	AAAP P	
RC_AA449450_at	AAAP P	

Or from

Homo sapiens (clones "MDP4," MDP7) microsomal dipeptidase (MDP) "mRNA," complete cds	**J05257**
"Homo sapiens reg gene ""homologue,"" complete cds"	**L08010**

H.sapiens mRNA for prepro-alpha2(I) collagen	**Z74616**
"Human S-adenosylhomocysteine hydrolase (AHCY) ""mRNA,"" complete cds"	**M61832**
Transcription Factor Iiia	**HG4312-HT4582**
Human gene for melanoma growth stimulatory activity (MGSA)	**X54489**
Human stromelysin-3 mRNA	**X57766**
CDC25Hu2=cdc25+ homolog "[human," "mRNA," 3118 nt]	S78187
Human mRNA for cripto protein	X14253
Human transformation-sensitive protein (IEF SSP 3521) "mRNA," complete cds	**M86752**
Human complement component 2 (C2) gene allele b	L09708
H.sapiens mRNA for ITBA2 protein	X92896
H.sapiens encoding CLA-1 mRNA	Z22555
"Human fibroblast growth factor receptor 4 (FGFR4) ""mRNA,"" complete cds"	L03840
"""Fibronectin,"" Alt. Splice 1"	HG3044-HT3742
tyk2	X54667
Human mRNA for B-myb gene	X13293
"Human phosphofructokinase (PFKM) ""mRNA,"" complete cds"	U24183
Human pre-B cell enhancing factor (PBEF) "mRNA," complete cds	U02020
Human SH2-containing inositol 5-phosphatase (hSHIP) "mRNA," complete cds	U57650
Human interleukin 8 (IL8) "gene," complete cds	**M28130**
"Human lamin B receptor (LBR) ""mRNA,"" complete cds"	L25931
H.sapiens mRNA for protein tyrosine phosphatase	Z48541
Human mRNA for unc-18 "homologue," complete cds	D63851
H.sapiens mRNA for Zn-alpha2-glycoprotein	X59766 Z25521
"Human asparagine synthetase ""mRNA,"" complete cds"	M27396
Human hepatitis delta antigen interacting protein A (dipA) "mRNA," complete cds	U63825
Human splicesomal protein (SAP 61) "mRNA," complete cds	U08815
Human protein kinase C-binding protein RACK7 "mRNA," partial cds	U48251
Human MAC30 "mRNA," 3' end	L19183
Human thrombospondin 2 (THBS2) "mRNA," complete cds	L12350
"Human nicotinamide N-methyltransferase (NNMT) ""mRNA,"" complete cds"	U08021
H.sapiens mRNA for type I interstitial collagenase	X54925
Human cytochrome b561 gene	U29463
Human H19 RNA "gene," complete cds (spliced in silico)	**M32053**
Human collagen type XVIII alpha 1 (COL18A1) "mRNA," partial cds	L22548
Human clone 23733 "mRNA," complete cds.	U79274

In another embodiment the probes consists of the sequences identified above.

The hybridization may be tested in vitro at conditions corresponding to in vivo conditions. Typically, hybridization conditions are of low to moderate stringency. These conditions favour specific interactions between completely complementary sequences, but allow some non-specific interaction between less than perfectly matched sequences to occur as well. After hybridization, the nucleic acids can be "washed" under moderate or high conditions of stringency to dissociate duplexes that are bound together by some non-specific interaction (the nucleic acids that form these duplexes are thus not completely complementary).

As is known in the art, the optimal conditions for washing are determined empirically, often by gradually increasing the stringency. The parameters that can be changed to affect stringency include, primarily, temperature and salt concentration. In general, the lower the salt concentration and the higher the temperature, the higher the stringency. Washing can be initiated at a low temperature (for example, room temperature) using a solution containing a salt concentration that is equivalent to or lower than that of the hybridization solution. Subsequent washing can be carried out using progressively warmer solutions having the same salt concentration. As alternatives, the salt concentration can be lowered and the temperature maintained in the washing step, or the salt concentration can be lowered and the temperature increased. Additional parameters can also be altered. For example, use of a destabilizing agent, such as formamide, alters the stringency conditions.

In reactions where nucleic acids are hybridized, the conditions used to achieve a given level of stringency will vary. There is not one set of conditions, for example, that will allow duplexes to form between all nucleic acids that are 85% identical to one another; hybridization also depends on unique features of each nucleic acid. The length of the sequence, the composition of the sequence (for example, the content of purine-like nucleotides versus the content of pyrimidine-like nucleotides) and the type of nucleic acid (for example, DNA or RNA) affect hybridization. An additional consideration is whether one of the nucleic acids is immobilized (for example, on a filter).

An example of a progression from lower to higher stringency conditions is the following, where the salt content is given as the relative abundance of SSC (a salt solution containing sodium chloride and sodium citrate; 2X SSC is 10-fold more concentrated than 0.2X SSC). Nucleic acids are hybridized at 42°C in 2X SSC/0.1% SDS (sodium dodecylsulfate; a detergent) and then washed in 0.2X SSC/0.1% SDS at room temperature (for conditions of low stringency); 0.2X SSC/0.1% SDS at 42°C (for conditions of moderate stringency); and 0.1X SSC at 68°C (for conditions of high stringency). Washing can be carried out using only one of the conditions given, or each of the conditions can be used (for example, washing for 10-15 minutes each in the order listed above). Any or all of the washes can be repeated. As mentioned above, optimal conditions will vary and can be determined empirically.

In another aspect a method of reducing tumoregeneicity relates to the use of antibodies against an expression product of a cell from the biological tissue. The antibodies may be produced by any suitable method, such as a method comprising the steps of

obtaining expression product(s) from at least one gene said gene being expressed as defined above for oncogenes,

immunising a mammal with said expression product(s) obtaining antibodies against the expression product.

Use

The methods described above may be used for producing an assay for diagnosing a biological condition in animal tissue, or for identification of the origin of a piece of tissue.

Furthermore, the invention relates to the use of a peptide as defined above for preparation of a pharmaceutical composition for the treatment of a biological condition in animal tissue.

Furthermore, the invention relates to the use of a gene as defined above for preparation of a pharmaceutical composition for the treatment of a biological condition in animal tissue.

Also, the invention relates to the use of a probe as defined above for preparation of a pharmaceutical composition for the treatment of a biological condition in animal tissue.

Gene delivery therapy

The genetic material discussed above for may be any of the described genes or functional parts thereof. The constructs may be introduced as a single DNA molecule encoding all of the genes, or different DNA molecules having one or more genes. The constructs may be introduced simultaneously or consecutively, each with the same or different markers.

The gene may be linked to the complex as such or protected by any suitable system normally used for transfection such as viral vectors or artificial viral envelope, liposomes or micellas, wherein the system is linked to the complex.

Numerous techniques for introducing DNA into eukaryotic cells are known to the skilled artisan. Often this is done by means of vectors, and often in the form of nucleic acid encapsidated by a (frequently virus-like) proteinaceous coat. Gene delivery systems may be applied to a wide range of clinical as well as experimental applications.

Vectors containing useful elements such as selectable and/or amplifiable markers, promoter/enhancer elements for expression in mammalian, particularly human, cells, and which may be used to prepare stocks of construct DNAs and for carrying out transfections are well known in the art. Many are commercially available.

Various techniques have been developed for modification of target tissue and cells in vivo. A number of virus vectors, discussed below, are known which allow transfection and random integration of the virus into the host. See, for example, Dubensky et al. (1984) Proc. Natl. Acad. Sci. USA 81:7529-7533; Kaneda et al., (1989)

Science 243:375-378; Hiebert et al. (1989) Proc. Natl. Acad. Sci. USA 86:3594-3598; Hatzoglu et al., (1990) J. Biol. Chem. 265:17285-17293; Ferry et al. (1991) Proc. Natl. Acad. Sci. USA 88:8377-8381. Routes and modes of administering the vector include injection, e.g intravascularly or intramuscularly, inhalation, or other parenteral administration.

Advantages of adenovirus vectors for human gene therapy include the fact that recombination is rare, no human malignancies are known to be associated with such viruses, the adenovirus genome is double stranded DNA which can be manipulated to accept foreign genes of up to 7.5 kb in size, and live adenovirus is a safe human vaccine organisms.

Another vector which can express the DNA molecule of the present invention, and is useful in gene therapy, particularly in humans, is vaccinia virus, which can be rendered non-replicating (U.S. Pat. Nos. 5,225,336; 5,204,243; 5,155,020; 4,769,330).

Based on the concept of viral mimicry, artificial viral envelopes (AVE) are designed based on the structure and composition of a viral membrane, such as HIV-1 or RSV and used to deliver genes into cells in vitro and in vivo. See, for example, U.S. Pat. No. 5,252,348, Schreier H. et al., J. Mol. Recognit., 1995, 8:59-62; Schreier H et al., J. Biol. Chem., 1994, 269:9090-9098; Schreier, H., Pharm. Acta Helv. 1994, 68:145-159; Chander, R et al. Life Sci., 1992, 50:481-489, which references are hereby incorporated by reference in their entirety. The envelope is preferably produced in a two-step dialysis procedure where the "naked" envelope is formed initially, followed by unidirectional insertion of the viral surface glycoprotein of interest. This process and the physical characteristics of the resulting AVE are described in detail by Chander et al., (supra). Examples of AVE systems are (a) an AVE containing the HIV-1 surface glycoprotein gp160 (Chander et al., supra; Schreier et al., 1995, supra) or glycosyl phosphatidylinositol (GPI)-linked gp120 (Schreier et al., 1994, supra), respectively, and (b) an AVE containing the respiratory syncytial virus (RSV) attachment (G) and fusion (F) glycoproteins (Stecenko, A. A. et al., Pharm. Pharmacol. Lett. 1:127-129 (1992)). Thus, vesicles are constructed which mimic the natural membranes of enveloped viruses in their ability to bind to and deliver materials to cells bearing corresponding surface receptors.

AVEs are used to deliver genes both by intravenous injection and by instillation in the lungs. For example, AVEs are manufactured to mimic RSV, exhibiting the RSV F surface glycoprotein which provides selective entry into epithelial cells. F-AVE are loaded with a plasmid coding for the gene of interest, (or a reporter gene such as CAT not present in mammalian tissue).

The AVE system described herein in physically and chemically essentially identical to the natural virus yet is entirely "artificial", as it is constructed from phospholipids, cholesterol, and recombinant viral surface glycoproteins. Hence, there is no carry-over of viral genetic information and no danger of inadvertant viral infection. Construction of the AVEs in two independent steps allows for bulk production of the plain lipid envelopes which, in a separate second step, can then be marked with the desired viral glycoprotein, also allowing for the preparation of protein cocktail formulations if desired.

Another delivery vehicle for use in the present invention are based on the recent description of attenuated Shigella as a DNA delivery system (Sizemore, D. R. et al., Science 270:299-302 (1995), which reference is incorporated by reference in its entirety). This approach exploits the ability of Shigellae to enter epithelial cells and escape the phagocytic vacuole as a method for delivering the gene construct into the cytoplasm of the target cell. Invasion with as few as one to five bacteria can result in expression of the foreign plasmid DNA delivered by these bacteria.

A preferred type of mediator of nonviral transfection in vitro and in vivo is cationic (ammonium derivatized) lipids. These positively charged lipids form complexes with negatively charged DNA, resulting in DNA charged neutralization and compaction. The complexes endocytosed upon association with the cell membrane, and the DNA somehow escapes the endosome, gaining access to the cytoplasm. Cationic lipid:DNA complexes appear highly stable under normal conditions. Studies of the cationic lipid DOTAP suggest the complex dissociates when the inner layer of the cell membrane is destabilized and anionic lipids from the inner layer displace DNA from the cationic lipid. Several cationic lipids are available commercially. Two of these, DMRI and DC-cholesterol, have been used in human clinical trials. First generation cationic lipids are less efficient than viral vectors. For delivery to lung, any inflammatory responses accompanying the liposome administration are reduced by

changing the delivery mode to aerosol administration which distributes the dose more evenly.

Drug screening

Genes identified as changing in various stages of colorectal cancer can be used as markers for drug screening. Thus by treating colorectal cancer cells with test compounds or extracts, and monitoring the expression of genes identified as changing in the progression of colorectal cancers, one can identify compounds or extracts which change expression of genes to a pattern which is of an earlier stage or even of normal colorectal mucosa.

The following are non-limiting examples illustrating the present invention.

Experimentals

We have used two different approaches to identify tumor suppressors, oncogenes and classifiers. The first approach was based on a spreadsheet approach in which we used the fold change and the pattern of expression being present or absent in the different preparations of RNA. The second approach was based on a mathematical approach in which we used correlation to a predefined profile as selection criteria based on Pearsons correlation coefficient.

Examples

Example 1

Quantification of gene expression using microarrays

Material

Colon tumor and normal oral resection edge biopsies were sampled from each patient after informed consent was obtained, and after removal of the necessary amount of tissue for routine pathological examination. Number of Tissue examined was: Normal resection edge 6, Dukes A, 5; B, 6; C, 6; D,4. The six normal tissue samples were all from Dukes A individuals.

RNA from Different tumors of the same stage were combined to form each pool. Five isuch pools were prepared as Normal pool, Dukes A pool, Dukes B pool, Dukes C pool, Dukes D pool. All tumors and normal tissue specimens were from the sigmoid or upper rectum.

Preparation of mRNA

Total mRNA was isolated using the RNAzol B RNA isolation method (WAK-Chemie Medical GMBH). Poly (A) + RNA was isolated by an oligo-dT selection step (Oligotex mRNA kit from Qiagen).

Preparation of cRNA

One µg mRNA was used as starting material for the cDNA preparation. The first and second strand cDNA synthesis was performed using the SuperScript Choice System (Life Technologies) according to the manufacturer's instructions, except that an oligo-dT primer containing a T7 RNA polymerase promoter site was used. Labeled cRNA was prepared using the MEGAscript In Vitro Transcription kit (Ambion). Biotin labeled CTP and UTP (Enzo) was used in the reaction together with unlabeled NTP's. Following the IVT reaction, the unincorporated nucleotides were removed using RNeasy columns (Qiagen).

Array hybridization and scanning

Ten µg of cRNA was fragmented at 94°C for 35 min. In a fragmentation buffer containing 40 mM Tris-acetate pH 8.1, 100 mM KOAc, 30 mM MgOAc. Prior to hybridization, the fragmented cRNA in a 6xSSPE-T hybridization buffer (1 M NaCL, 10 mM Tris pH 7.6, 0.005% Triton) was heated to 95 °C for 5 min. And subsequently to 40°C for 5 min. Before loading onto an Affymetrix probe array cartridge. The probe array was then incubated for 16 h at 40 °C at constant rotation (60 rpm). The washing and staining procedure was performed in the Affymetrix Fluidics Station. The probe array was exposed to 10 washes in 6X SSPE-T at 25°C followed by 4 washes in 0.5xSSPE-T at 50°C. The biotinylated cRNA was stained with a streptavidin-phycoerythrin conjugate, 10 µg/ml (Molecular Probes, Eugene, OR) in 6xSSPE-T for 30 min. at 25°C followed by 10 washes in 6xSSPE-T at 25°C. The

prove arrays were scanned at 560 nm using a confocal laser scanning microscope with an argon ion laser as the excitation source (made for Affymetrix by Molecular Dynamics). Following this scan, the array was incubated with an anti-avidin antibody and an biotinylated anti-immunoglobulin, and the streptavidin-phycoerythrin step was repeated.

The readings from the quantitative scanning were analyzed by the Affymetrix Gene Expression Analysis Software.

Normalization of data

To compare samples, normalization of the data was necessary. For that purpose we compared scaling to total GAPDH intensity (sum of 3´, middle, 5`probe sets) of 7000 units with scaling to a total array intensity (global scaling) of 281850 units (averaging 150 units per probe set). Both gave similar results with scaling factors that differed less than ten percent in a set of experiments. Based on this we chose the global scaling for all experiments.

Example 2

Change of transcript level during the progression of colon cancer

Biopsies from human colon tumors were analyzed as pools of tumors representing the different stages in the progression of the colon cancer disease. A total of 4 tumor pools were used, each pool made by combining four to six tumors (see materials and methods). To generate a normal reference material, we pooled biopsiesfrom normal colon mucosa from six volunteers.

From the biopsies RNA was extracted, reverse transcribed to cDNA and the cDNA transcribed into labelled cRNA, that was incubated on the array cartridges followed by scanning and scaling to a global array intensity amounting to 150 units per probe set. The scaling made it possible to compare individual experiments to each other. To verify the reproducibility, double determinations were made in selected cases and showed a good correlation.

The software GeneArray Analysis Suite 3.1 from Affymetrix, Inc. Was used to analyse the array data. In this software, increased levels indicate that the transcript is

either up-regulated at the stated level or turned on de novo reaching a given fold above the background level. Decreased levels in a similar way indicate reduction or loss of transcript. Alterations of a single transcript during the progression of the colon cancer disease can follow several different pathways . Some of the transcript changes reflect the transition from normal cells to tumor cells, Others an increase in malignancy from Dukes A to Dukes B.

Example 2

A. ***Finding Classifiers of and predictors etc. of colorectal cancer based on a spreadsheat approach.***

We used a spreadsheat to sort genes based on different parameters obtained from the Affymetrix analysis software.

The mRNA expression analysis on the AFFYMETRIX ARRAYs resulted in 42.843 datasets identifying individual genes (table I) or EST´s (table II),altogether. These were obtained from the 6.8k Arrays (7.129 datasets) and the EST ARRAYs (35.714 datasets)

Description of the Sorting Procedure for the spreadsheat sorting,

Per dataset the following was listed,
Probe Set No., Present or absent in Normal tissue or the different Duke´s types, gene name or homoogy or number, "AvgDiff" which is the level of expression, "Abs Call" which determines if the gene is present (P) or absent (A) , "Diff call" which determines the alteration as increasing (I) or decreasing (D), "fold change" the fold change from normal tissue expression level,, and the "sort score" which determines the likelihood that it is real changes (if above 0.5).

The following steps were performed,

1. exclude data if "Probe Set" is an AFFX-marker (58/array or sub-array)
2. exclude data if "Diff Call" in all 4 comparisons is "NC" *(no change)*
3. exclude data if "Abs Call" in all 4 comparisons is "A" *(absent)*
4. exclude data if three "Abs call" are "NC" and one is "MI or MD"
5. select data with absolute value of |sort score| arbitrarily set to >= 0,5

(At this step the sorting resulted in the following number of genes sorted as being of importance, 908 Genes (12,7 %) and 4155 ESTs (11,6 %)

6. sort according to pattern of Abs Calls (e.g. PAAAA = lost from N to tumour Duke ABCD)
7. select data with Avg Diff of >= 300 (500 for some ESTs) and /or fold change >= 3 (>= 5 for some ESTs)
   Number of genes sorted out as being of interest after this final sorting, ≈ 130 Genes (1,8 %), ≈ 240 ESTs (0,7 %)

The following tables show the genes (Table I) and EST´+s (Table II) that were identified by this approach, analyzing the hu 6.8K FI gene array. First a list of the potential tumor suppressors, then a list of the potential oncogenes, finally a list of genes that can be used to classify the different Dukes Stages. Genes that are in bold are those that we find are of the utmost interest.

The table (Table III) that follow this section are based on the hu EST arrays Hu35k Sub A,B,C,D. These are also divided into EST´s that are supposed to be expressed from tumor suppressors, and oncogenes, as well as from genes that can be used as classifiers of the different Dukes stages. The most intersting Est´s are shown in bold.

Table I

Fold Change in comparison to normal SUPPRESSOR CLASSIFIER

Gene name	Acc No	Avg Diff	Avg Diff	
CRC classifier genes lost PAAAA or PPAAA		N	A	B
"Human chromogranin A ""mRNA,"" complete cds"	J03915	831	lost	lost
Human adipsin/complement factor D "mRNA," complete cds	M84526	822	lost	lost
Homo sapiens MLC-1V/Sb isoform gene	M24248	799	lost	lost
Human aminopeptidase N/CD13 mRNA encoding aminopeptidase "N," complete cds	M22324	657	lost	lost
H.sapiens MT-1l mRNA	X76717	650	lost	lost
H.sapiens GCAP-II gene	Z70295	572	lost	lost
Human somatostatin I gene and flanks	J00306	516	lost	lost
Human YMP "mRNA," complete cds	U52101	459	lost	lost
H.sapiens mRNA for beta subunit of epithelial amiloride-sensitive sodium channel	X87159	439	lost	lost
Human K12 protein precursor "mRNA," complete cds	U77643	429	121	lost
Human sulfate transporter (DTD) "mRNA," complete cds	U14528	397	lost	lost
Human transcription factor hGATA-6 "mRNA," complete cds.	U66075	337	lost	lost
H.sapiens SCAD "gene," exon 1 and joining features	Z80345	326	lost	lost
Human S-lac lectin L-14-II (LGALS2) gene	M87860	301	lost	lost
Human mRNA for protein tyrosine phosphatase	D15049	277	43	lost
H.sapiens mRNA for tetranectin	X64559	235	lost	lost
Human 11kd protein "mRNA," complete cds	U28249	233	47	lost
Human anti-mullerian hormone type II receptor precursor "gene," complete cds	U29700	223	lost	lost
Human heparin binding protein (HBp17) "mRNA," complete cds	M60047	218	lost	lost
Human ADP-ribosylation factor (hARF6) "mRNA," complete cds	M57763	209	lost	lost
beta -ADD=adducin beta subunit 63 kda isoform/membrane skeleton protein, beta -ADD=adducin beta subunit 63 kda isoform/membrane skeleton protein (alternatively spliced, exon 10 to 13 region) [human, Genomic, 1851 nt, segment 3 of 3].	S81083	188	lost	lost
Zinc Finger Protein Znf155	HG4243-HT4513	186	lost	lost
Human glucagon "mRNA," complete cds	J04040	182	25	lost
H.sapiens mRNA for hair "keratin," hHb5	X99140	158	lost	lost
Human tubulin-folding cofactor E "mRNA," complete cds	U61232	150	lost	lost
Human integrin alpha-3 chain "mRNA," complete cds	M59911	126	lost	lost
Human NACP gene	U46901	123	lost	lost
H.sapiens mRNA for flavin-containing monooxygenase 5 (FMO5)	Z47553	110	lost	lost
Human mRNA for ATF-a transcription factor	X52943	104	lost	lost
H.sapiens intestinal VIP receptor related protein mRNA	X77777	93	lost	lost

Gene name	Acc No	Avg Diff	fold change to N
Only A Classifier		N	A
Homo sapiens SKB1Hs "mRNA," complete cds. /gb=AF015913 /ntype=RNA	AF015913	188	Lost
Mucin (Gb:M22406)	HG1067-HT1067	501	Lost
Human platelet activating factor "acetylhydrolase," brain "isoform," 45 kDa subunit (LIS1) gene	U72342	114	Lost
Homosapiens ERK activator kinase (MEK2) mRNA	L11285	1470	-5,2
Human 20-kDa myosin light chain (MLC-2) "mRNA," complete cds	J02854	2047	-4,5
H.sapiens lysosomal acid phosphatase gene (EC 3.1.3.2) Exon 1 (and joined CDS).	X15525	285	-4,4
Human mRNA for matrix Gla protein	X53331	1069	-4,2
H.sapiens mRNA for diacylglycerol kinase	X62535	362	-3,5
Human heat shock protein (hsp 70) gene, complete cds.	M11717	405	-3,2
Human TRPM-2 protein gene	M63379	1594	-3
Only B Classifier		N	B
Human gene for mitochondrial acetoacetyl-CoA thiolase	D10511	198	lost
Human mRNA for transcription factor "AREB6," complete	D15050	232	lost

cds			
Human mRNA for KIAA0248 "gene," partial cds	D87435	374	lost
Homo sapiens (clone CC6) NADH-ubiquinone oxidoreductase subunit "mRNA," 3' end cds	L04490	683	lost
Human phosphoglucomutase 1 (PGM1) "mRNA," complete cds	M83088	1096	lost
Homo sapiens guanylin "mRNA," complete cds	M97496	4983	lost
"Human trans-Golgi p230 ""mRNA,"" complete cds"	U41740	131	lost
H.sapiens mRNA for vacuolar proton "ATPase," subunit D	X71490	414	lost
H.sapiens mRNA for 3-hydroxy-3-methylglutaryl coenzyme A synthase	X83618	2196	lost
Human mRNA for KIAA0018 "gene," complete cds	D13643	377	-7,7
"Mucin ""1,"" ""Epithelial,"" Alt. Splice 9"	HG371-HT26388	3296	-4,1
H.sapiens mRNA for L-3-hydroxyacyl-CoA dehydrogenase	X96752	252	-3

Only C Classifier		N	C
Homo sapiens colon mucosa-associated (DRA) "mRNA," complete cds	L02785	2978	Lost
Human Ig J chain gene	M12759	2193	Lost
Human selenium-binding protein (hSBP) "mRNA," complete cds. /gb=U29091 /ntype=RNA	U29091	1849	Lost
H.sapiens mRNA for sigma 3B protein	X99459	722	Lost
Human ERK1 mRNA for protein serine/threonine kinase	X60188	576	Lost
Human mRNA for mitochondrial 3-oxoacyl-CoA "thiolase," complete cds	D16294	529	Lost
"Biliary ""Glycoprotein,"" Alt. Splice ""5,"" A"	HG2850-HT4814	489	Lost
Human AQP3 gene for aquaporine 3 (water "channel)," partail cds	AB001325	413	Lost
Human CD14 mRNA for myelid cell-specific leucine-rich glycoprotein	X13334	413	Lost
Human thioredoxin "mRNA," nuclear gene encoding mitochondrial "protein," complete cds	U78678	411	Lost
Human mitochondrial ATPase coupling factor 6 subunit (ATP5A) "mRNA," complete cds	M37104	373	Lost
"Human MHC class II HLA-DP light chain ""mRNA,"" complete cds"	M57466	327	Lost
Human mRNA for early growth response protein 1 (hEGR1)	X52541	281	Lost
Human mRNA for mitochondrial 3-ketoacyl-CoA thiolase beta-subunit of trifunctional "protein," complete cds	D16481	268	Lost
Homo sapiens laminin-related protein (LamA3) "mRNA," complete cds	L34155	252	Lost
H.sapiens mRNA for selenoprotein P	Z11793	232	Lost
Human hkf-1 "mRNA," complete cds	D76444	211	Lost
Homo sapiens nuclear domain 10 protein (ndp52) "mRNA," complete cds	U22897	150	Lost
Human X104 "mRNA," complete cds	L27476	149	Lost
H. sapiens cDNA for RFG	X77548	130	Lost
H.sapiens mRNA for Progression Associated Protein	Y07909	128	Lost
Human liver "2,4-dienoyl-CoA" reductase "mRNA," complete cds	U49352	101	Lost
Human A33 antigen precursor "mRNA," complete cds	U79725	1650	-6,9
H.sapiens pS2 protein gene	X52003	4298	-6
Human RASF-A PLA2 "mRNA," complete cds	M22430	4983	-5,8
Homo sapiens pstI mRNA for pancreatic secretory inhibitor (expressed in neoplastic tissue).	Y00705	344	-3,1
Human CO-029	M35252	3500	-3

Only D Classifier		N	D
Human complement component C3 "mRNA," alpha and beta "subunits," complete cds	K02765	744	lost
H.sapiens mRNA for adenosine "triphosphatase," calcium	Z69881	439	lost
Human skeletal muscle LIM-protein SLIM1 "mRNA," complete cds	U60115	281	lost
Human platelet-derived growth factor receptor alpha	M21574	187	lost

(PDGFRA) "mRNA," complete cds			
Human mRNA for KIAA0247 "gene," complete cds	D87434	172	lost
Human mRNA for KIAA0171 "gene," complete cds	D79993	151	lost
Human Down syndrome critical region protein (DSCR1) "mRNA," complete cds	U28833	150	lost
Human Ki nuclear autoantigen "mRNA," complete cds	U11292	125	lost

AB Classifier		N	A	B
Homo sapiens chromosome 16 BAC clone CIT987SK-815A9 complete sequence.	AF001548	3513	-3,6	-4,3
Human mRNA for ATP synthase alpha "subunit," complete cds	D14710	3580	-3,8	-5,6

BC Classifier		N	B	C
Human mRNA for IgG Fc binding "protein," complete cds	D84239	3755	-19,3	-7,1
H.sapiens mRNA for carcinoembryonic "antigen," CGM2	X98311	2456	-12	-6,5
"Homo sapiens (clone lamda-hPEC-3) phosphoenolpyruvate carboxykinase (PCK1) ""mRNA,"" complete cds"	L05144	2630	-7,6	-14,7
Human 11-beta-hydroxysteroid dehydrogenase type 2 "mRNA," complete cds	U26726	1865	-7,1	-4,7
"Human intestinal mucin (MUC2) ""mRNA,"" complete cds"	L21998	7803	-5,5	-4,2
Human mRNA for KIAA0106 "gene," complete cds	D14662	766	-4,7	-3,2
metallothionein	V00594	5417	-4	-6,3

Table I(cont.)

Fold Change in comparison to normal — Oncogene CLASSIFIER

Gene name	Acc No	Avg Diff	Avg Diff	
CRC classifier genes gained APPPP or AAPPP		A	B	
Homo sapiens (clones "MDP4," MDP7) microsomal dipeptidase (MDP) "mRNA," complete cds	J05257	1606	1403	gained
"Homo sapiens reg gene ""homologue,"" complete cds"	L08010	1165	294	gained
H.sapiens mRNA for prepro-alpha2(I) collagen	Z74616	1003	905	gained
"Human S-adenosylhomocysteine hydrolase (AHCY) ""mRNA,"" complete cds"	M61832	882	817	gained
Transcription Factor IIia	HG4312-HT4582	837	948	gained
Human gene for melanoma growth stimulatory activity (MGSA)	X54489	731	330	gained
Human stromelysin-3 mRNA	X57766	643	1116	gained
CDC25Hu2=cdc25+ homolog "[human," "mRNA," 3118 nt]	S78187	603	627	gained
Human mRNA for cripto protein	X14253	532	293	gained
Human transformation-sensitive protein (IEF SSP 3521) "mRNA," complete cds	M86752	529	866	gained
Human complement component 2 (C2) gene allele b	L09708	515	625	gained
H.sapiens mRNA for ITBA2 protein	X92896	444	459	gained
H.sapiens encoding CLA-1 mRNA	Z22555	422	549	gained
"Human fibroblast growth factor receptor 4 (FGFR4) ""mRNA,"" complete cds"	L03840	359	276	gained
"""Fibronectin,"" Alt. Splice 1"	HG3044-HT3742	354	261	gained
tyk2	X54667	336	352	gained
Human mRNA for B-myb gene	X13293	333	322	gained
"Human phosphofructokinase (PFKM) ""mRNA,"" complete cds"	U24183	296	426	gained
Human pre-B cell enhancing factor (PBEF) "mRNA," complete cds	U02020	276	242	gained
Human SH2-containing inositol 5-phosphatase (hSHIP)	U57650	254	315	gained

"mRNA," complete cds			
Human interleukin 8 (IL8) "gene," complete cds	**M28130**	**251**	**609 gained**
"Human lamin B receptor (LBR) ""mRNA,"" complete cds"	L25931	239	193 gained
H.sapiens mRNA for protein tyrosine phosphatase	Z48541	228	151 gained
Human mRNA for unc-18 "homologue," complete cds	D63851	217	198 gained
H.sapiens mRNA for Zn-alpha2-glycoprotein	X59766	215	156 gained
	Z25521	215	127 gained
"Human asparagine synthetase ""mRNA,"" complete cds"	M27396	212	195 gained
Human hepatitis delta antigen interacting protein A (dipA) "mRNA," complete cds	U63825	211	231 gained
Human spliceosomal protein (SAP 61) "mRNA," complete cds	U08815	157	201 gained
Human protein kinase C-binding protein RACK7 "mRNA," partial cds	U48251	129	71 gained
Human MAC30 "mRNA," 3' end	L19183	128	224 gained
Human thrombospondin 2 (THBS2) "mRNA," complete cds	L12350	111	126 gained
"Human nicotinamide N-methyltransferase (NNMT) ""mRNA,"" complete cds"	U08021	107	261 gained
H.sapiens mRNA for type I interstitial collagenase	X54925	105	123 gained
Human cytochrome b561 gene	U29463	85	85 gained
Human H19 RNA "gene," complete cds (spliced in silico)	**M32053**	**72**	**4498 gained**
Human collagen type XVIII alpha 1 (COL18A1) "mRNA," partial cds	L22548	67	275 gained
Human clone 23733 "mRNA," complete cds.	U79274	absent	162 gained

Gene name	Acc No	Avg Diff	fold change to N
Only A Classifier		A	
Human migration inhibitory factor-related protein 8 (MRP8) "gene," complete cds	M21005	120	GAINED
Human acyloxyacyl hydrolase "mRNA," complete cds	M62840	130	GAINED
Human PEP19 (PCP4) "mRNA," complete cds	U52969	174	GAINED
H.sapiens Humig mRNA	X72755	118	GAINED
H.sapiens PISSLRE mRNA	X78342	125	GAINED
H.sapiens mRNA for twist "protein," partial. /gb=Y11180 /ntype=RNA	Y11180	121	GAINED
Human mRNA for TGF-beta superfamily "protein," complete cds	AB000584	1372	3,5
Human mRNA for "MSS1," complete cds	D11094	292	3,1
Human complement factor B "mRNA," complete cds	L15702	2082	3,3
"Homo sapiens GTP-binding protein (RAB2) ""mRNA,"" complete cds"	M28213	289	3,1
Human translational initiation factor 2 beta subunit (eIF-2-beta) "mRNA," complete cds	M29536	956	4,1
Human E16 "mRNA," complete cds	M80244	278	3,8
IEX-1=radiation-inducible immediate-early gene "[human," "placenta," mRNA "Partial," 1223 nt]	S81914	1531	3,6
Human CDC16Hs "mRNA," complete cds	U18291	244	6,1
Human DD96 "mRNA," complete cds	U21049	625	3,2
Human (memc) "mRNA," 3'UTR. /gb=U30999 /ntype=RNA	U30999	256	3,8
"Human ubiquitin-conjugating enzyme (UBE2I) ""mRNA,"" complete cds"	U45328	448	10,6
"Human fetal brain glycogen phosphorylase B ""mRNA,"" complete cds"	U47025	2349	3,7
"Human BTG2 (BTG2) ""mRNA,"" complete cds"	U72649	527	5,2
Human jun-B mRNA for JUN-B protein	X51345	1350	4,6

Only B Classifier		B	
Human adipocyte lipid-binding "protein," complete cds	J02874	268	GAINED
Human A1 protein "mRNA," complete cds	U29680	102	GAINED
Human LGN protein "mRNA," complete cds	U54999	110	GAINED
Human skeletal muscle LIM-protein SLIM2 "mRNA," partial cds	U60116	109	GAINED
Human mRNA for alpha1-acid glycoprotein (orosomucoid)	X02544	156	GAINED
Human mRNA for fibronectin receptor alpha subunit	X06256	46	GAINED

H.sapiens P1-Cdc21 mRNA	X74794	278	GAINED
H.sapiens mRNA for fibulin-2	X82494	284	GAINED
H.sapiens 5T4 gene for 5T4 Oncofetal antigen	Z29083	152	GAINED
Homo sapiens mRNA for osteoblast specific factor 2 (OSF-2os)	D13666	324	7,6
Mac25	HG987-HT987	2772	3,3
"Human lysozyme ""mRNA,"" complete cds with an Alu repeat in the 3' flank"	J03801	920	3,7
Human metalloproteinase (HME) "mRNA," complete cds	L23808	794	7,4
Human alpha-1 collagen type IV gene, exon 52.	M26576	610	4,9
Human lumican "mRNA," complete cds	U21128	1105	4,1
Human mRNA for fibronectin (FN precursor)	X02761	4181	5,5
Human mRNA fragment for elongation factor TU (N-terminus). /gb=X03689 /ntype=RNA	X03689	3515	3,1
Human mRNA for type IV collagen alpha -2 chain	X05610	1531	3
Human mRNA for collagen VI alpha-1 C-terminal globular domain	X15880	2062	3,5
"H.sapiens," gene for Membrane cofactor protein	X59405	272	3,4
H.sapiens SOD-2 gene for manganese superoxide dismutase. /gb=X65965 /ntype=DNA /annot=exon	X65965	234	3,1
H.sapiens NMB mRNA	X76534	338	3,3
H.sapiens vimentin gene	Z19554	3472	3,2

Only C Classifier		C	
Ribosomal Protein L39 Homolog	HG2874-HT3018	102	GAINED
Homo sapiens (clone d2-115) kappa opioid receptor (OPRK1) "mRNA," complete cds	L37362	168	GAINED
Human kell blood group protein mRNA	M64934	143	GAINED
	U73167	374	GAINED
Human cancellous bone osteoblast mRNA for serin protease with IGF-binding "motif," complete cds	D87258	504	3,4
Human interferon-inducible protein 27-Sep "mRNA," complete cds	J04164	7717	3,8
"Human sickle cell beta-globin ""mRNA,"" complete cds"	M25079	3090	4,6
	M29277	1588	3,7
"Human spermidine synthase ""mRNA,"" complete cds"	M34338	866	4,1
Human copine I "mRNA," complete cds	U83246	2079	3,7

Only D Classifier		D	
Homo sapiens FRG1 "mRNA," complete cds	L76159	73	GAINED
Human cyclin protein "gene," complete cds	M15796	149	GAINED
Human U2 small nuclear RNA-associated B" antigen "mRNA," complete cds	M15841	194	GAINED
Human mRNA export protein Rae1 (RAE1) "mRNA," complete cds.	U84720	193	GAINED
Human protease-activated receptor 3 (PAR3) "mRNA," complete cds.	U92971	142	GAINED
H.sapiens mRNA for mediator of receptor-induced toxicity	X84709	200	GAINED
H.sapiens RFXAP mRNA	Y12812	230	GAINED
Human mRNA for "Qip1," complete cds	AB002533	8881	2,7
Human mRNA for transferrin receptor	X01060	557	3
"metastasis-associated gene ""[human,"" highly metastatic lung cell subline ""Anip[937],"" mRNA ""Partial,"" 978 nt]"	S79219	216	4

AB Classifier			A	B
Human chaperonin 10 "mRNA," complete cds	U07550	50	4,1	3,3
H.sapiens RING4 cDNA	X57522	73	4,9	5,4
H.sapiens genes TAP1, TAP2, LMP2, LMP7 and DOB.	X66401	134	3,2	3,1
H.sapiens mRNA for alpha 4 protein	Y08915	96	3,7	3,6
Homo sapiens interleukin-1 receptor-associated kinase (IRAK) "mRNA," complete cds	L76191	285	3,1	3,1
"Human von Willebrand factor ""mRNA,"" 3' end"	M10321	84	3,7	4,1
Human chromosome segregation gene homolog CAS "mRNA," complete cds	U33286	86	4,8	3,6
Human Bruton's tyrosine kinase-associated protein-135	U77948	68	3,4	4,9

"mRNA," complete cds.				
"Human KH type splicing regulatory protein KSRP ""mRNA,"" complete cds."	U94832	52	3,2	3,2
H.sapiens ADE2H1 mRNA showing homologies to SAICAR synthetase and AIR carboxylase of the purine pathway (EC "6.3.2.6," EC 4.1.1.21)	X53793	40	3	3,1

BC Classifier		N	B	C
""Globin,"" Beta"	HG1428-HT1428	504	3,1	4,3
"Human alpha-1 collagen type I ""gene,"" 3' end"	M55998	2706	3,1	3,7
H.sapiens mRNA for SOX-4 protein	X70683	130	4,5	4,5
"Human mRNA for collagen binding protein ""2,"" complete cds"	D83174	131	8,1	6,1
Human SPARC/osteonectin "mRNA," complete cds	J03040	358	6,1	3,9
Human PRAD1 mRNA for cyclin	X59798	263	3,3	3,4

ABC Classifier		N	A	B	C
Human transforming growth factor-beta induced gene product (BIGH3) "mRNA," complete cds	M77349	426	4,7	6,7	4,4
"Human breast epithelial antigen BA46 ""mRNA,"" complete cds"	U58516	169	3,3	3,2	4,2
	X57351	460	4,8	3,5	3,7
H.sapiens NGAL gene	X99133	327	8,3	3,1	4,8
Human mRNA for MDNCF (monocyte-derived neutrophil chemotactic factor)	**Y00787**	**87**	**5**	**9,2**	**13,4**
H.sapiens EF-1delta gene encoding human elongation factor-1-delta	Z21507	198	4,4	6,8	4,5
H.sapiens mRNA for prepro-alpha1(I) collagen	Z74615	285	5	8,2	6,1
Nuclear Factor Nf-Il6	HG3494-HT3688	246	4,3	4,4	4,2
	U29175	62	4,3	3,6	4,4

ABCD Classifier		N	A	B	C	D
"HNL=neutrophil lipocalin ""[human,"" ovarian cancer cell line ""OC6,"" mRNA ""Partial,"" 534 nt]. /gb=S75256 /ntype=RNA"	S75256	361	8,8	4,3	7,7	9

Table II

EST candidates	Tumor stages	*unknown genes, data found after blast search*																							
Probe Set	NABCD	homologous to	EST name	Avg Diff N	Abs Call N	Avg Diff A	Abs Call A	Diff Call Avs N	Fold Change Avs N	Sort Score Avs N	Avg Diff B	Abs Call B	Diff Call Bvs N	Fold Change Bvs N	Sort Score Bvs N	Avg Diff C	Abs Call C	Diff Call Cvs N	Fold Change Cvs N	Sort Score Cvs N	Avg Diff D	Abs Call D	Diff Call Dvs N	Fold Change Dvs N	Sort Score Dvs N
LOST from N to tumour (tumour suppressors)																									
RC_H04768_at	PAAAA	*chrom 15 no homology*	yj51f03.s1 Homo sapiens cDNA clone 152285 3'.	1078	P	127	A	D	-8,4	-7,99	104	A	D	-10,2	-9,57	133	A	D	-9,3	-9,54	46	A	D	~-7,8	-0,22
RC_Z39652_at	PAAAA	*Y14593 APM-1 gene adipocyte-specific secretory protein; chrom 1q21.3-q23*	H. sapiens partial cDNA sequence; clone c-1fg03.	959	P	-157	A	D	~-19,7	-15,28	-212	A	D	~-22,9	-18,17	-269	A	D	~-21,5	-16,51	16	A	D	~-7,0	-0,53
RC_H30270_at	PAAAA	**chrom 18 PAAAA in colon & bladder *no homology***	yp42e02.s1 Homo sapiens cDNA clone 190106 3' similar to contains MSR1 repetitive element ;.	941	P	366	A	D	-4,1	-3,11	287	A	D	-7,1	-6,36	240	A	D	-8,1	-7,28	114	A	D	~-5,7	-8,11
RC_T47089_s_at	PAAAA	tenascin-X; tenascin-X precursor; unidentified protein	yb52b08.s1 Homo sapiens cDNA clone	815	P	-49	A	D	~-31,4	-18,16	79	A	D	-10,4	-8,51	15	A	D	~-21,5	-14,77	-42	A	D	~-21,8	-4,93

			74775 3' similar to gb:M25813 FIBRI-NOGEN-LIKE PROTEIN (HUMAN).																							
RC_W31906_at	PAAAA	secretagogin; dJ501N12.8 (putative protein) chrom 6	zc76c03.s1 Pancre-atic Islet Homo sapiens cDNA clone 328228 3'.	736	P	243	A	D	-4,8	-3,12	148	A	D	-2,6	-1,18	263	A	D	-2,8	-1,34	147	A	D	~-17,0	-0,4
RC_AA279803_at	PAAAA	*chrom 2 no homology*	zs92a11.s1 NCI_CGAP_GCB1 Homo sapiens cDNA clone IMAGE:704924 3'.	643	P	164	A	D	-3,9	-2,35	217	A	D	-3	-1,4	91	A	D	-4,3	-2,73	161	A	D	-11,1	-12,84
RC_R01646_at	PAAAA	chrom *13q32.1-33.3 ; AL159152 ; homology to mouse Pcbp1 - poly(rC)-binding protein 1*	ye79f11.s1 Homo sapiens cDNA clone 123981 3'.	607	P	-75	A	D	~-17,7	-13,83	92	A	D	-10,1	-8,86	67	A	D	-13,8	-11,32	-37	A	D	~-6,5	-5,64
RC_AA099820_at	PAAAA	*BAC clone AC016778*	zk87c05.s1 Soares pregnant uterus NbHPU Homo sapiens cDNA clone 489800 3'.	587	P	58	A	D	~-9,5	-6,53	17	A	D	~-10,0	-6,91	38	A	D	~-9,8	-6,76	-5	A	D	~-4,7	-0,52
AA319615_at	PAAAA	secretory carrier membrane protein;	EST21862 Adrenal	568	P	-100	A	D	~-34,7	-15,7	-198	A	D	~-39,6	-17,5	-103	A	D	~-34,8	-15,8	-225	A	D	~-35,	-14,5

		secretory carrier membrane protein 2; chrom 15	gland tumor Homo sapiens cDNA 5' end.							6					2					2				4	3
H07011_at	PAAAA	**tetraspan NET-6 mRNA; transmembrane 4 superfamily; chrom 7**	yl81e01.r1 Homo sapiens cDNA clone 44466 5'.	324	P	123	A	D	-2,6	-0,78	5	A	D	~-14,9	-6,63	122	A	D	-2,6	-0,79	193	A	D	-1,7	-9,87
RC_T68873_f_at	PPAAA		yc30f03.s1 Homo sapiens cDNA clone 82205 3' similar to gb:J00272_rna1 Human metallothionein-II pseudogene (HUMAN);contains L1 repetitive element ;.	3837	P	611	P	D	-6	-10,26	269	A	D	-13,5	-22,23	14	A	D	~-61,8	-46,51	440	A	D	-8,3	-14,71
RC_T40995_f_at	PPAAA		ya15e08.s3 Homo sapiens cDNA clone 61574 3'.	1973	P	841	P	D	-2,7	-2,24	-8	A	D	~-73,5	-38,45	533	A	D	-4,3	-5,18	856	A	D	-4,2	-4,98
RC_H81070_f_at	PPAAA		yu60h05.s1 Homo sapiens cDNA clone 230553 3' similar to	1683	P	469	P	D	-4,3	-4,92	-228	A	D	~-87,0	-43,73	-100	A	D	~-60,5	-38,36	-183	A	D	~-64,2	-39,64

			gb:X64177 H.sapiens mRNA for metallothi-onein (HUMAN);.																						
RC_N30796_at	PPPAA		yw65d03.s1 Homo sapiens cDNA clone 257093 3'.	1338	P	584	P	D	-2,7	-1,48	217	P	D	-5	-4,48	345	A	D	-3,7	-3,04	711	A	D	-2,6	-1,48
RC_W37778_f_at	PPAAA		zc13b12.s1 Soares parathy-roid tumor NbHPA Homo sapiens cDNA clone 322175 3' similar to contains LTR2.t3 LTR2 repetitive element ;.	945	P	371	P	D	-3,5	-2,53	174	A	D	-6,5	-6,21	237	A	D	-4,6	-3,92	280	A	D	-4,3	-3,58
RC_R70212_s_at	PPAAA		yj80d09.s1 Homo sapiens cDNA clone 155057 3' similar to gb:U05259_rna1 MB-1 MEM-BRANE GLY-COPRO-TEIN PRECUR-SOR	718	P	227	P	D	-3,1	-1,57	84	A	D	-8,5	-6,7	109	A	D	-7,4	-6,17	115	A	D	-6,2	-4,77

			(HUMAN);.																						
RC_AA426330_at	PPAAA		zw11h09.s1 Soares NhHMPuS1 Homo sapiens cDNA clone 769025 3'.	676	P	164	P	D	-4,1	-2,75	36	A	D	~-19,0	-11,89	-227	A	D	~-23,3	-14,18	-23	A	D	~-19,4	-20,17
RC_N33927_s_at	PPAAA		yv25e09.s1 Homo sapiens cDNA clone 243784 3'.	599	P	312	P	D	-1,9	-0,46	151	A	D	-4	-2,33	222	A	D	-4,1	-2,44	109	A	D	~-3,8	-2,11
RC_T90190_s_at	PPAAA		yd38f12.s1 Homo sapiens cDNA clone 110543 3'.	574	P	102	P	D	-4,8	-2,79	33	A	D	-14,8	-8,64	56	A	D	-8,8	-5,67	170	A	D	-3,4	-1,73
RC_AA447145_at	PPPAA		zw93f08.s1 Soares total fetus Nb2HF89w Homo sapiens cDNA clone 784551 3'.	374	P	123	P	D	-3	-1,14	64	P	D	-5,9	-3,21	60	A	D	-6,2	-3,44	130	A	D	-2,9	-1,04
RC_H75860_at	PPAAA		yu60g09.s1 Homo sapiens cDNA clone 230560 3'.	355	P	70	P	D	-4	-1,64	13	A	D	~-11,1	-6,17	5	A	D	~-10,8	-5,85	29	A	D	~-8,6	-7,11
RC_T71132_s_at	PPAAA		yd34a02.s1 Homo sapiens cDNA clone 110090 3' similar to gb:L13288	325	P	131	P	D	-2,6	-0,81	54	A	D	-6,4	-3,4	73	A	D	~-8,5	-4,61	9	A	D	~-8,6	-4,57

			VASOACTIVE INTESTINAL POLYPEPTIDE RECEPTOR 1 PRECURSOR (HUMAN);.																						
GAINED from N to tumour (oncogene)																									
RC_AA609013_s_at	APPPP	microsomal dipeptidase (also on 6.8k); chrom 16	af05f12.s1 Soares testis NHT Homo sapiens cDNA clone 1030799 3' similar to gb:J05257 MICROSOMAL DIPEPTIDASE PRECURSOR (HUMAN);.	421	A	2176	P	I	5,1	6,42	3117	P	I	6,7	10,33	3163	P	I	7,5	12,38	2447	P	I	5,8	8,12
RC_AA232508_at	APPPP	CGI-89 protein; unnamed protein product; hypothetical protein	zr28d08.s1 Stratagene NT2 neuronal precursor 937230 Homo sapiens cDNA clone 664719 3'.	516	A	2511	P	I	4,5	5,56	2138	P	I	4,1	4,71	1942	P	I	3,1	2,5	3179	P	I	5,7	8,6
RC_AA428964_at	APPPP	serine protease-like protease; serine protease homolog=NES1;	zw19b02.s1 Soares ovary	395	A	559	P	I	1,4	0,13	1894	P	I	5,2	6,55	969	P	I	3,1	2,19	1037	P	I	4,3	3,56

		normal epithelial cell-specific 1	tumor NbHOT Homo sapiens cDNA clone 769707 3' similar to gb:X57025_rna1 INSULIN-LIKE GROWTH FACTOR IA PRE-CURSOR (HUMAN);.																						
RC_T52813_s_at	APPPP	dJ28O10.2 (G0S2 (PUTATIVE LYM-PHOCYTE G0/G1 SWITCH PROTEIN 2; chrom 1	ya79h10.s1 Homo sapiens cDNA clone 67939 3' similar to gb:M69199 PUTA-TIVE LYM-PHOCYTE G0/G1 SWITCH PROTEIN (HUMAN).	191	A	471	P	I	3,1	1,2	1827	P	I	8,4	9,84	1597	P	I	7,3	8,03	604	P	I	1,4	0,14
RC_AA075642_at	APPPP	gp-340 variant protein; DMBT1/8kb.2 protein	zm88a11.s1 Strata-gene ovarian cancer (#937219) Homo sapiens cDNA clone 544988 3'.	264	A	3364	P	I	13	20,97	893	P	I	9	7,83	5271	P	I	20,1	34,59	7085	P	I	9,7	23,8

RC_AA007218_at	APPPP	chrom 13 no homology	13cDNA54-3.seq Soares infant brain 1NIB Homo sapiens cDNA clone HY18-131 3'.	156	A	902	P	I	5,8	4,88	878	P	I	5,6	4,65	884	P	I	5,7	4,71	711	P	I	~5,4	4,47
RC_N33920_at	APPPP	ubiquitin-like protein FAT10; diubiquitin; dJ271M21.6 (Diubiquitin); chrom 6	yv25b11.s1 Homo sapiens cDNA clone 243741 3'.	15	A	1173	P	I	34,5	22,41	811	P	I	~27,4	17,37	425	P	I	~12,5	7,18	524	P	I	~5,8	3,73
RC_N71781_at	APPPP	KIAA1199 protein, chrom 15	yz94e06.s1 Homo sapiens cDNA clone 290722 3'.	9	A	575	P	I	~16,7	10,24	775	P	I	~22,0	13,85	305	P	I	~9,4	4,81	579	P	I	~6,6	4,59
RC_R67275_s_at	APPPP	alpha-1 (type XI) collagen precursor; collagen, type XI, alpha 1; collagen type XI alpha-1 isoform A; chrom 1	yh01f11.s2 Homo sapiens cDNA clone 41676 3' similar to gb:J04177 COLLAGEN ALPHA 1(XI) CHAIN PRECURSOR (HUMAN);.	17	A	403	P	I	~10,9	6,01	699	P	I	~17,6	10,87	468	P	I	~11,8	6,7	411	P	I	~4,3	2,06
RC_W80763_at	APPPP	hypothetical protein; chrom 17	zd83g04.s1 Soares fetal heart NbHH19W Homo	193	A	571	P	I	3,6	1,97	658	P	I	4,2	2,67	789	P	I	5	3,82	747	P	I	3,9	2,5

			sapiens cDNA clone 347286 3'.																						
RC_AA443793_at	APPPP	*chrom 7p22 AC006028 BAC clone*	zw86e11.s 1 Soares total fetus Nb2HF8 9w Homo sapiens cDNA clone 783884 3'.	232	A	781	P	I	3,5	2,19	594	P	I	3,6	2,27	847	P	I	3,7	2,51	473	P	I	1,4	0,12
RC_AA034499_s_at	APPPP	ZNF198 protein; zinc finger protein; FIM protein; Cys-rich protein; zinc finger protein 198; chrom 13	zk23c04.s 1 Soares pregnant uterus NbHPU Homo sapiens cDNA clone 471366 3'.	128	A	589	P	I	4,7	2,97	594	P	I	4,7	2,98	536	P	I	4,3	2,56	464	P	I	~3,2	1,47
RC_AA035482_at	APPPP	*chrom 5; AK022505 clone; CalcineurinB (weakly similar)*	zk27b07.s 1 Soares pregnant uterus NbHPU Homo sapiens cDNA clone 471733 3'.	260	A	562	P	I	1,7	0,3	506	P	I	1,9	0,39	572	P	I	2,2	0,67	592	P	I	3	1,37
RC_AA024482_at	APPPP	hypothetical protein; unnamed protein product; chrom 17	ze76a01.s 1 Soares fetal heart NbHH19W Homo sapiens cDNA clone 364872 3'.	-28	A	828	P	I	~44,0	19,93	445	P	I	~26,6	12,37	360	P	I	~21,9	10,07	443	P	I	~22,7	11,3
RC_H93021_at	APPPP	*chrom 2 ; XM_004890 peptidylprolyl isomera-*	yv06a03.s 1 Homo	274	A	345	P	I	3	1,29	436	P	I	3,7	2,12	384	P	I	3,3	1,58	590	P	I	2,7	1,3

		se A (cyclophilin A)	sapiens cDNA clone 241900 3' similar to gb:X52851 _rna1 PEP-TIDYL-PROLYL CIS-TRANS ISOME-RASE A (HUMAN);.																						
RC_AA427737_at	APPPP	*no homology*	zw30g12.s1 Soares ovary tumor NbHOT Homo sapiens cDNA clone 770854 3'.	145	A	339	P	I	2,3	0,6	419	P	I	2,9	1,07	515	P	I	3,5	1,77	461	P	I	3,2	1,37
RC_AA417078_at	APPPP	*chrom 7q31; AF017104 clone*	zu13c02.s1 Soares testis NHT Homo sapiens cDNA clone 731714 3'.	59	A	405	P	I	3,2	1,21	411	P	I	3,3	1,34	462	P	I	3,7	1,71	337	P	I	2,7	0,83
M29873_s_at	APPPP	**cytochrome P450-IIB (hIIB3) ; 19q13.1-q13.2**	Human cytochrome P450-IIB (hIIB3) mRNA, complete cds.	19	A	640	P	I	~32,3	15,15	372	P	I	5,2	2,7	760	P	I	~38,3	17,75	400	P	I	5,5	3,08
RC_H27498_f_at	AAPPP		yl57a08.s1 Homo sapiens cDNA	2557	A	1129	A	NC	-2,3	-1,52	6246	P	I	2,4	2,87	9295	P	I	3,6	7,91	7876	P	I	3,1	5,35

			clone 162326 3' similar to gb:S55735 IG ALP-HA-1 CHAIN C REGION (HUMAN);.																						
RC_T92363_s_at	AAPPP		ye19h06.s1 Homo sapiens cDNA clone 118235 3'.	68	A	255	A	NC	3,7	1,37	730	P	I	10,7	8,3	238	P	I	3,5	1,18	311	P	I	~2,4	0,53
RC_N89910_at	AAAPP		zb22f11.s1 Soares fetal lung NbHL19W Homo sapiens cDNA clone 302829 3' similar to SW:PGT_RAT Q00910 PROSTAG-LANDIN TRANS-PORTER ;.	205	A	226	A	NC	1,1	0,01	149	A	NC	1,1	0,01	301	P	I	1,5	0,12	250	P	NC	-2,3	-0,74
RC_W60516_at	AAAPP		zc99f02.s1 Pancreatic Islet Homo sapiens cDNA clone 339291 3' similar to contains Alu repeti-	391	A	285	A	NC	-1,1	-0,02	328	A	NC	1	0	437	P	I	1,1	0,02	404	P	NC	-2,7	-1,51

			tive element;.																						
RC_AA219699_at	AAAPP		zr03d01.s1 Stratagene NT2 neuronal precursor 937230 Homo sapiens cDNA clone 650401 3' similar to contains Alu repetitive element;.	15	A	110	A	NC	~2,6	0,37	109	A	MI	~2,6	0,35	114	P	I	~2,7	0,4	293	P	I	~2,6	0,57
RC_AA449450_at	AAAPP		zx05e04.s1 Soares total fetus Nb2HF8 9w Homo sapiens cDNA clone 785598 3'.	237	A	315	A	NC	1,3	0,07	526	A	NC	2,2	0,65	411	P	I	1,7	0,27	395	P	NC	-1,7	-0,3
A classifiers [illegible]																									
Gained in Duke A and Inc; NC in other Dukes;																									
Avg Diff in A > 300																									
RC_AA599199_at	APAAA	ALU seq.	ag34a11.s1 Jia bone marrow stroma Homo sapiens cDNA clone 1091420 3' similar to contains Alu repeti-	4	A	833	P	I	~24,1	15,25	284	A	NC	~8,8	4,36	176	A	NC	~5,8	2,11	568	A	NC	-2,1	-0,83

			tive element;contains element TAR1 repetitive element ;.																						
RC_R12694_at	APAAA	unnamed protein product BAA91641, chrom 10	yf40f05.s1 Homo sapiens cDNA clone 129345 3' similar to contains Alu repetitive element;.	115	A	361	P	I	3	1,02	200	A	NC	1,9	0,27	208	A	NC	1,8	0,22	213	A	NC	~1,6	0,11
RC_H91325_s_at	APAAA	aldolase B; aldolase B (aa 1-364); chrom 9	yu96g02.s1 Homo sapiens cDNA clone 241106 3' similar to gb:X02747 FRUCTOSE-BISPHOSPHATE ALDOLASE B (HUMAN);.	92	A	318	P	I	3,4	1,33	157	A	NC	1,7	0,16	71	A	NC	-1,3	-0,03	96	A	NC	1	0
RC_N51709_at	APAAA	chrom X	yy72e04.s1 Homo sapiens cDNA clone 279102 3'.	466	A	440	P	I	-1,1	-0,01	378	A	NC	-1,2	-0,05	473	A	NC	1	0	435	A	NC	-3	-2,05
RC_N72610_at	APAAA	-	za46h03.s1 Homo sapiens cDNA	502	A	531	P	I	1,1	0,01	338	A	NC	1,5	0,13	618	A	NC	1,2	0,06	373	A	NC	-3,7	-3,23

			clone 295637 3'.																						
Lost in Duke A and Dec; NC in other Dukes																									
Avg Diff in N > 300																									
RC_N32411_f_at	PAPPP	Myc-associated zinc-finger protein of human islet; chrom 16	yw83b08.s1 Homo sapiens cDNA clone 258807 3' similar to gb:M94046 Human zinc finger protein (HUMAN);.	4153	P	1319	A	D	-3,5	-5,06	3491	P	NC	-1,2	-0,11	4943	P	NC	1,2	0,12	3403	P	NC	-1,3	-0,27
RC_AA243858_at	PAPPP	KIAA0882 protein	zr68c02.s1 Soares NhHMPu S1 Homo sapiens cDNA clone 668546 3'.	389	P	77	A	D	-5	-2,67	548	P	NC	1,4	0,13	334	P	NC	-1,2	-0,03	322	P	NC	-3,4	-2,36
RC_AA486283_at	PAPPP	ras-like protein; ras-related C3 botulinum toxin substrate; dJ20J23	ab35h03.s1 Stratagene HeLa cell s3 937216 Homo sapiens cDNA clone 842837 3' similar to gb:M29870 RAS-RELATED C3 BOTULINUM TOXIN SUB-	4858	P	720	A	D	-6,2	-12,02	3548	P	NC	-1,4	-0,33	5135	P	NC	1,1	0,02	5504	P	NC	1,1	0,07

			STRATE 2 (HUMAN);.																					
RC_AA490930_at	PAPPP	chrom 18; *KIAA1468 protein*	aa46e04.s 1 NCI_CGA P_GCB1 Homo sapiens cDNA clone IMAGE:82 3998 3'.	552	P	242	A	D	-2,3	-0,72	214	P	NC	-2,6	-0,97	225	P	N C	-2,5	-0,86	175	P	N C	-3,2	-1,48
PPPPP; Inc in Duke A; NC in other Dukes																								
Avg Diff N >= 200; Fold change N to A >= 3x																								
RC_N69263_at	PPPPP	*chrom 10; AK026414 clone (only 108 nt hom)*	za38e10.s 1 Homo sapiens cDNA clone 294858 3'.	314	P	1239	P	I	3,4	2,35	909	P	NC	2,5	1,12	681	P	N C	1,9	0,43	660	P	N C	1,8	0,35
RC_T15817_f_at	PPPPP	iNOS, inducible nitric oxide synthase	IB1935 Infant brain, Bento Soares Homo sapiens cDNA 3'end similar to H. sapiens inducible nitric oxide synthase mRNA.	484	P	1182	P	I	3,4	2,45	606	P	NC	1,3	0,06	865	P	N C	1,8	0,44	753	P	N C	-1,3	-0,11
PPPPP; Dec in Duke A; NC in other Dukes																								
Avg Diff N >= 200; Fold change N to A >= 3x																								
RC_H54088_s_at	PPPPP	ribosomal protein L41	yq88g11.s 1 Homo sapiens cDNA	5317	P	1302	P	D	-4,1	-7,26	4157	P	NC	-1,3	-0,22	6623	P	N C	1,2	0,21	5372	P	N C	1	0

			clone 202916 3'.																						
RC_H59052_f_at	PPPPP	fungal sterol-C5-desaturase homolog; ORF; thymosin beta-4	yr40g11.s1 Homo sapiens cDNA clone 207812 3' similar to gb:M17733 THY-MOSIN BETA-4 (HUMAN);.	5090	P	1086	P	D	-4,2	-7,05	4077	P	NC	-1,2	-0,18	5764	P	NC	1,1	0,08	5216	P	NC	1	0,01
RC_R49198_s_at	PPPPP	-	yg58h09.s1 Homo sapiens cDNA clone 37141 3' similar to gb:X69150 40S RI-BOSO-MAL PROTEIN S18 (HU-MAN);.	4836	P	1115	P	D	-4,3	-7,61	3144	P	NC	-1,5	-0,58	4785	P	NC	-1	0	5478	P	NC	1,1	0,07
RC_T73572_f_at	PPPPP	ferritin L-chain; L apoferritin	yc36c10.s1 Homo sapiens cDNA clone 82770 3' similar to gb:M10119 FERRI-TIN LIGHT CHAIN (HUMAN);.	5727	P	1853	P	D	-3,1	-4,59	5148	P	NC	-1,1	-0,06	6119	P	NC	1,1	0,03	5457	P	NC	-1	-0,02
RC_AA477483_at	PPPPP	no matching est	zu44h02.s1 Soares ovary tumor	4494	P	1034	P	D	-3,8	-5,51	3186	P	NC	-1,4	-0,37	4175	P	NC	-1,1	-0,03	4052	P	NC	-1,1	-0,05

			NbHOT Homo sapiens cDNA clone 740883 3' similar to SW:NU3M_HUMAN P03897 NADH-UBI-QUINONE OXIDO-REDUC-TASE CHAIN 3 ;.																						
B-classifiers [illegible]																									
Gained in Duke B and Inc; NC in other Dukes																									
Avg Diff in B > 300																									
RC_T67463_s_at	AAPAA	cathepsin O2; X; K	yd12c04.s1 Homo sapiens cDNA clone 66918 3' similar to gb:X12451 CATHEP-SIN L PRECUR-SOR (HUMAN);.	132	A	240	A	NC	1,8	0,24	713	P	I	5,4	3,97	226	A	NC	1,7	0,19	275	A	NC	~1,3	0,05
RC_W94688_at	AAPAA	perilipin	ze04f12.s1 Soares fetal heart NbHH19W Homo sapiens cDNA clone 358031 3'.	139	A	137	A	NC	-1	0	393	P	I	2,8	1	84	A	NC	-1	0	92	A	NC	-1,5	-0,09
RC_AA128743_at	AAPAA	*Z97200 PAC chrom*	zk95d02.s	402	A	384	A	NC	4,3	2,1	734	P	I	1,8	0,43	98	A	N	1,1	0,02	310	A	N	-	-0,07

		1q24; PMX1 homeobox gene	1 Soares pregnant uterus NbHPU Homo sapiens cDNA clone 490563 3'.															C					C	1,3	
RC_AA236547_at	AAPAA	*no homology*	zs39a03.s 1 Soares NhHMPu S1 Homo sapiens cDNA clone 687532 3'.	223	A	-151	A	NC	-2,3	-0,48	402	P	I	1,8	0,3	285	A	NC	1,3	0,05	191	A	NC	-2,1	-0,6
RC_AA255567_at	AAPAA	angiopoietin-related protein-2; angiopoietin-like 2	zr85e11.s 1 Soares NhHMPu S1 Homo sapiens cDNA clone 682508 3'.	118	A	160	A	NC	1,3	0,05	408	P	I	3,4	1,5	207	A	NC	1,7	0,2	235	A	NC	-1,4	-0,1
RC_AA421258_at	AAPAA	-	zu06a01.s 1 Soares testis NHT Homo sapiens cDNA clone 731016 3'.	338	A	171	A	NC	-2	-0,38	382	P	I	1,1	0,02	236	A	NC	-1,1	-0,01	269	A	NC	-3,8	-2,9
Lost in Duke B and Dec; NC in other Dukes																									
Avg Diff in N > 300																									
RC_D59847_at	PPAPP	proSAAS; granin-like neuroendocrine peptide precursor	Human fetal brain cDNA 3'-end GEN-070G07.	864	P	668	P	NC	-1,3	-0,1	336	A	D	-2,6	-1,2	308	P	NC	-1,9	-0,56	423	P	NC	-1,5	-0,22
RC_F05038_at	PPAPP	polyamine modulated factor-1; polyamine	H. sapiens partial	1683	P	1460	P	NC	-1,2	-0,05	972	A	D	-1,9	-0,84	1644	P	NC	-1	0	1068	P	NC	-4,4	-7,65

		modulated factor 1	cDNA sequence; clone c-1gb09.																						
RC_N41059_at	PPAPP	chrom 3	yy53h01.s1 Homo sapiens cDNA clone 277297 3'.	350	P	334	P	NC	-1	0	246	A	D	-1,4	-0,11	378	P	NC	1,1	0,01	287	P	NC	-3,4	-2,28
RC_T23460_at	PPAPP	chrom 3; *IFNAR2 21q22.11*	seq3026 Homo sapiens cDNA clone Hy18-Ch13-Charon40-cDNA-76 3'.	613	P	560	P	NC	-1,1	-0,01	402	A	D	-2	-0,53	570	P	NC	-1,3	-0,08	318	P	NC	-1,9	-0,47
RC_W42789_at	PPAPP	chrom 8 *AF268037 C8ORF4 protein (C8ORF4) chrom 8 ORF*	zc25a06.s1 Soares senescent fibroblasts NbHSF Homo sapiens cDNA clone 323314 3'.	310	P	234	P	NC	-1,3	-0,07	29	A	D	-5,6	-2,73	129	P	NC	-2,4	-0,61	489	P	NC	1,6	0,21
RC_AA460017_at	PPAPP	*BAC clone chrom 16*	zx49h08.s1 Soares testis NHT Homo sapiens cDNA clone 795615 3'.	1370	P	703	P	NC	-1,9	-0,73	725	A	D	-1,9	-0,66	666	P	NC	-2,1	-0,85	952	P	NC	-1,4	-0,23
RC_AA482127_at	PPAPP	KIAA1142 protein	zv43g10.s1 Soares ovary tumor NbHOT Homo	356	P	288	P	NC	-1,2	-0,04	138	A	D	-2,6	-0,78	211	P	NC	-1,7	-0,23	332	P	NC	-1,1	-0,01

			sapiens cDNA clone 756450 3'.																						
RC_AA504806_at	PPAPP	*chrom 2 AF052107 clone 23620 mRNA sequence*	aa64a10.s1 NCI_CGAP_GCB1 Homo sapiens cDNA clone IMAGE:825690 3'.	421	P	384	P	NC	-1,4	-0,12	135	A	D	-4,5	-2,02	351	P	NC	-1,2	-0,04	414	P	NC	-3,5	-2,64
Inc in Duke B; NC in other Dukes																									
Avg Diff N >= 200; Fold change N to A >= 3x																									
RC_AA386386_s_at	PPPPP	-	EST81368 Prostate gland I Homo sapiens cDNA 3' end similar to similar to prolyl 4-hydroxylase, beta subunit/protein disulfide isomerase/thyroid hormone-binding protein.	1672	P	2253	P	NC	1,4	0,3	4776	P	I	3	3,89	1559	P	NC	-1,1	-0,02	1731	P	NC	1,1	0,04
RC_AA452549_at	PPPPP	PRO1659; hypothetical protein chrom 11	zx35f04.s1 Soares total fetus Nb2HF8 9w Homo sapiens cDNA	400	P	368	P	NC	-1,1	-0,01	1255	P	I	3,1	2,22	422	P	NC	1,1	0,01	257	P	NC	-1,6	-0,17

			clone 788479 3'.																						
Dec in Duke B; NC in other Dukes																									
Avg Diff N >= 200; Fold change N to B >= 3x																									
RC_T90037_at	PPPPP	unnamed protein product, chrom 4	ye13d01.s 1 Homo sapiens cDNA clone 117601 3'.	948	P	568	P	NC	-1,7	-0,36	204	P	D	-4,6	-3,72	395	P	NC	-2,4	-1,07	466	P	NC	-2	-0,69
RC_AA432130_at	PPPPP	KIAA0867 protein, chrom 12	zw69f10.s 1 Soares testis NHT Homo sapiens cDNA clone 781483 3' similar to contains Alu repetitive element;contains element PTR7 repetitive element ;.	1007	P	475	P	NC	-2,1	-0,8	226	P	D	-5,4	-5,23	534	P	NC	-1,9	-0,56	437	P	NC	-6,4	-9,82
C-classifiers [illegible]																									
Gained in Duke C and Inc; NC in other Dukes																									
Avg Diff in C > 300																									
RC_D45556_at	AAAPA	*chrom 15; AL390085 clone*	Human adult lung 3'directed MboI cDNA, HUMGS02743, clone lg1160.	391	A	222	A	NC	-1,8	-0,28	407	A	NC	1	0	536	P	I	1,4	0,11	362	A	NC	-3	-1,89
RC_W86214_at	AAAPA		zh54a11.s	88	A	132	A	NC	1,6	0,12	305	A	NC	2,2	0,39	409	P	I	3,5	1,34	247	A	N	-	-0,1

			1 Soares fetal liver spleen 1NFLS S1 Homo sapiens cDNA clone 415868 3'.																					C	1,4	
RC#AA039439_s_at	AAAPA	*novel gene KIAA0134 protein 19q13.3*	zk39a11.s1 Soares pregnant uterus NbHPU Homo sapiens cDNA clone 485180 3'.	134	A	608	A	NC	3,8	1,94	320	A	NC	3,2	1,39	600	P	I	4,5	2,81	259	A	NC	~1,7	0,19
RC_AA128935_at	AAAPA		zo08e09.s1 Stratagene neuro epithelium NT2RAMI 937234 Homo sapiens cDNA clone 567112 3'.	162	A	270	A	NC	1,7	0,19	245	A	NC	1,5	0,12	416	P	I	2,6	0,84	412	A	NC	~1,8	0,26
RC_AA134158_s_at	AAAPA	class I homeodomain; homeobox protein, chrom 7	zo18b11.s1 Stratagene colon (#937204) Homo sapiens cDNA clone 587229 3' similar to gb:S41211 HOMEO-BOX	255	A	200	A	NC	3,7	1,17	273	A	NC	5	2,21	988	P	I	3,9	2,87	416	A	NC	-1,7	-0,34

			PROTEIN HOX-A10 (HUMAN);.																						
RC_AA232646_at	AAAPA	chrom 17, *AF266756 sphingosine kinase (SPHK1*	zr45f08.s1 Soares NhHMPu S1 Homo sapiens cDNA clone 666375 3'.	123	A	238	A	NC	1,9	0,3	601	A	NC	4,9	3,21	608	P	I	5	3,27	-18	A	NC	~1,1	0,01
RC_AA401184_at	AAAPA	*no homology*	zu52b06.s 1 Soares ovary tumor NbHOT Homo sapiens cDNA clone 741587 3'.	348	A	62	A	NC	~-1,2	-0,01	224	A	NC	~-2,2	-0,1	502	P	I	1,4	0,14	78	A	NC	~4,0	0,63
RC_AA436840_at	AAAPA		zv57g11.s 1 Soares testis NHT Homo sapiens cDNA clone 757796 3'.	399	A	-13	A	NC	~-6,1	-2	367	A	NC	1,4	0,1	458	P	I	1,7	0,3	57	A	NC	-1,5	-0,08
RC_AA488655_at	AAAPA		ab40c03.s 1 Strata-gene HeLa cell s3 937216 Homo sapiens cDNA clone 843268 3'.	-4	A	176	A	NC	~6,8	2,59	257	A	NC	~9,5	4,43	360	P	I	~9,6	5,32	124	A	NC	~4,1	1,01
Lost in Duke C and Dec; NC in other Dukes																									
Avg Diff in N > 300																									
RC_N30231_at	PPPAP	Lsm4 protein; U6	yw64c08.s	1496	P	885	P	NC	-2,2	-1,26	1161	P	NC	-1,3	-0,13	-978	A	D	~-	-36	808	P	N	-	-0,67

		snRNA-associated Sm-like protein LSm4; glycine-rich protein	1 Homo sapiens cDNA clone 257006 3' similar to contains Alu repetitive element;.																59,3				C	1,9	
RC_W73790_f_at	PPPAP	immunoglobulin-related protein 14.1; lambda L-chain C region; omega protein, chrom 22	zd50g12.s1 Soares fetal heart NbHH19W Homo sapiens cDNA clone 344134 3' similar to gb:M27749 IMMUNOGLOBULIN-RELATED 14.1 PROTEIN PRECURSOR (HUMAN);.	3320	P	1638	P	NC	-2	-1,27	728	P	NC	-4,2	-5,76	759	A	D	-4	-5,39	989	P	NC	-8,6	-23,38
RC_AA412184_at	PPPAP	*chrom 1p36; d89060 dolichyl-diphosphooligosaccharide-protein glycosyl-transferase*	zt93f09.s1 Soares testis NHT Homo sapiens cDNA clone 729929 3' similar to WP:EEED8.9 CE01893 ;.	714	P	570	P	NC	-1,4	-0,15	488	P	NC	-1,5	-0,18	437	A	D	-2	-0,54	545	P	NC	-1,4	-0,13
RC_AA521303_at	PPPAP	methionine adenosyl-	aa79f11.s	777	P	760	P	NC	-1	0	479	P	NC	-1,6	-0,29	338	A	D	-2,3	-0,87	741	P	N	-1	-0,01

		transferase regulatory beta subunit; dTDP-4-keto-6-deoxy-D-glucose 4-reductase, chrom 5	1 NCI_CGAP_GCB1 Homo sapiens cDNA clone IMAGE:827181 3'.																				C		
Inc in Duke C; NC in other Dukes																									
Avg Diff N >= 200; Fold change N to C >= 3x																									
RC_AA181902_at	PPPPP	*AC007201 on chrom 19 (only 80nt hom)*	zp63d06.s1 Stratagene endothelial cell 937223 Homo sapiens cDNA clone 624875 3' similar to contains Alu repetitive element;.	478	P	679	P	NC	1,6	0,29	614	P	NC	1,5	0,21	1347	P	I	3,1	2,3	565	P	NC	1,2	0,04
Dec in Duke C; NC in other Dukes																									
Avg Diff N >= 200; Fold change N to D >= 3x																									
RC_AA461174_at	PPPPP	*8p21.3-p22 AB020860 anti-oncogene*	zx70c04.s1 Soares total fetus Nb2HF8 9w Homo sapiens cDNA clone	327	P	198	P	NC	-1,5	-0,12	169	P	NC	-1,7	-0,23	84	P	D	-3,5	-1,3	170	P	NC	-1,7	-0,22

[illegible]			796806 3'.																							
AA393432_s_at	PPPPP	chrom 2, Unknown; unnamed protein product AAD20029	zt71a04.r1 Soares testis NHT Homo sapiens cDNA clone 727758 5'.	319	P	222	P	NC	-1,4	-0,11	159	P	NC	-2	-0,38	97	P	D	-3,3	-1,22	377	P	N C	-1,3	-0,05	
D-classifiers [illegible]																										
Gained in Duke D and Inc; NC in other Dukes																										
Avg Diff in D > 300																										
RC_N91920_at	AAAAP	*chrom 16p12-p11.2 ; XN_007994 retinoblastoma binding protein*	zb48e07.s1 Soares fetal lung NbHL19W Homo sapiens cDNA clone 306852 3'.	-76	A	58	A	NC	~4,7	0,62	123	A	NC	~6,6	1,88	-70	A	N C	~1,2	0,01	371	P	I	~6,9	3,52	
RC_AA621601_at	AAAAP	*chrom 17 XM_009868 RAB36 ARS oncogene family*	af47g08.s1 Soares total fetus Nb2HF8 9w Homo sapiens cDNA clone 1034846 3' similar to TR:G240986 G240986 LMW G-PROTEIN. ;.	-46	A	232	A	NC	~-3,5	-0,18	185	A	NC	~1,3	0,01	18	A	N C	~4,2	0,23	478	P	I	~12,5	5,32	
Lost in Duke D and Dec; NC in other Dukes																										
Avg Diff in N > 500																										
RC_R72886_s_at	PPPPA	KIAA0422; adenylyl cyclase type VI, chrom	yl10f04.s1 Homo	1768	P	880	P	NC	-2	-0,91	1052	P	NC	-1,7	-0,53	1528	P	N C	-1,5	-0,31	410	A	D	-4,4	-4,68	

		12	sapiens cDNA clone 157855 3'.																						
RC_AA026030_at	PPPPA	chrom 1	ze84d01.s1 Soares fetal heart NbHH19W Homo sapiens cDNA clone 365665 3' similar to PIR:A48764 A48764 calpain ;.	1478	P	1163	P	NC	-1,3	-0,11	1074	P	NC	-1,4	-0,19	1263	P	NC	-1,2	-0,06	514	A	D	-2,9	-2,02
RC_Z39006_at	PPPPA	hypothetical protein, chrom 17	H. sapiens partial cDNA sequence; clone c-0we07.	1251	P	691	P	NC	-1,4	-0,14	824	P	NC	-1,5	-0,28	876	P	NC	-1,4	-0,21	450	A	D	-2,8	-1,72
RC_AA435908_at	PPPPA	*chrom 19; ac011491 clone and 20 nt hom. RAB2, RAS oncogene family*	zt87a11.s1 Soares testis NHT Homo sapiens cDNA clone 729308 3'.	1173	P	494	P	NC	-1,6	-0,28	673	P	NC	-1,2	-0,04	394	P	NC	-2	-0,6	314	A	D	-7	-9,6
RC_AA057829_s_at	PPPPA	growth-arrest-specific protein; growth arrest-specific 6; AXL stimulatory factor, chrom 13	zl95c02.s1 Stratagene corneal stroma (#937222) Homo sapiens cDNA clone 512354 3' similar to TR:G401767	1136	P	534	P	NC	-1,5	-0,26	1572	P	NC	1,6	0,44	936	P	NC	-1	0	276	A	D	-2,7	-1,49

			G401767 GROWTH-ARREST-SPECIFIC PROTEIN. ;.																						
RC_R72087_at	PPPPA	*chrom 5 EST; hom to chrom 20 AL356652 clone*	yj87e06.s1 Homo sapiens cDNA clone 155746 3'.	923	P	768	P	NC	-1,2	-0,06	726	P	NC	-1,3	-0,09	971	P	NC	1,1	0,01	362	A	D	-2,5	-1,22
RC_H04242_at	PPPPA	ras related protein Rab5b; RAB5B, member RAS oncogene family	yj46c08.s1 Homo sapiens cDNA clone 151790 3'.	869	P	661	P	NC	-1,1	-0,01	365	P	NC	-1,4	-0,1	475	P	NC	-1,5	-0,17	298	A	D	-2,3	-0,86
RC_R97304_f_at	PPPPA	HLA-drb5; cell surface glycoprotein; MHC HLA-DR-beta chain precursor chrom 6	yq52c02.s1 Homo sapiens cDNA clone 199394 3' similar to gb:M33600 HLA CLASS II HISTO-COMPA-TIBILITY ANTIGEN, DR-1 BETA CHAIN (HUMAN);.	858	P	1822	P	NC	1,5	0,25	556	P	NC	-1,5	-0,25	680	P	NC	-1,3	-0,08	425	A	D	--24,3	-16,4
RC_N48609_at	PPPPA	*chrom 11; AC004584 chrom 17*	yy74f08.s1 Homo sapiens cDNA clone 279303 3'.	770	P	612	P	NC	-1,1	-0,02	884	P	NC	1,1	0,04	834	P	NC	1,1	0,01	265	A	D	-2,4	-0,99
RC_W86850_f_at	PPPPA	*chrom 22 ? X96924 mitochondrial citrate*	zh59d02.s1 Soares	644	P	273	P	NC	-1,4	-0,09	-63	P	NC	--23,9	-13,7	474	P	NC	-1,9	-0,59	-257	A	D	--22,	-14,3

		tranbsport region	fetal liver spleen 1NFLS S1 Homo sapiens cDNA clone 416355 3'.												5									8	
RC_AA130603_at	PPPPA	*ak024908 clone*	zo10a01.s1 Stratagene neuro epithelium NT2RAMI 937234 Homo sapiens cDNA clone 567240 3'.	640	P	568	P	NC	-1,1	-0,02	681	P	NC	1,1	0,01	598	P	NC	-1,1	-0,01	241	A	D	-2,7	-1,11
RC_AA479610_at	PPPPA	*singleton ak025344 clone*	zu31a12.s1 Soares ovary tumor NbHOT Homo sapiens cDNA clone 739582 3' similar to contains Alu repetitive element;.	640	P	571	P	NC	-1,1	-0,02	618	P	NC	-1	0	726	P	NC	1,1	0,03	337	A	D	-5,3	-6,14
RC_AA490593_at	PPPPA	*chrom 17 ? Synaptobrevin2 (VAMP2) AF135372*	aa47e09.s1 NCI_CGAP_GCB1 Homo sapiens cDNA clone IMAGE:82	540	P	536	P	NC	-1,3	-0,07	610	P	NC	-1,1	-0,02	518	P	NC	-1,4	-0,12	308	A	D	-6,5	-7,34

			4104 3'.																						
RC_AA064321_s_at	PPPPA	*6p21 HLA class i region; AC004202 clone*	zl68c01.s1 Stratagene colon (#937204) Homo sapiens cDNA clone 509760 3'.	509	P	472	P	NC	-1,1	-0,01	346	P	NC	-1,5	-0,15	441	P	NC	-1,2	-0,03	63	A	D	-- 3,6	-1,67
Inc in Duke D; NC in other Dukes																									
Avg Diff N >= 200; Fold change N to A >= 3x																									
D79052_s_at	PPPPP	Sec61 gamma	Human placenta cDNA 5'-end GEN-530B11.	772	P	680	P	NC	-1,3	-0,08	856	P	NC	-1	0	868	P	NC	1,1	0,03	3411	P	I	3,9	5,13
RC_T40439_s_at	PPPPP	U2 small nuclear ribonucleoprotein B"; dJ705D16.1	ya01c08.s1 Homo sapiens cDNA clone 60206 3' similar to SP:RU2B_HUMAN P08579 U2 SMALL NUCLEAR RIBONU-CLEOPROTEIN ,.	673	P	911	P	NC	1,2	0,06	934	P	NC	1,2	0,07	1125	P	NC	1,7	0,39	2813	P	I	4,2	5,48
RC_AA251829_at	PPPPP	CGI-29 protein	zs09c12.s1 NCI_CGAP_GCB1 Homo sapiens cDNA clone IMAGE:684694 3'.	202	P	670	P	NC	2,8	1,19	440	P	NC	2,2	0,57	366	P	NC	1,8	0,3	1445	P	I	6,7	7,21

Dec in Duke D; NC in other Dukes																									
Avg Diff N >= 500; Fold change N to D >= -5x																									
RC_D60328_at	PPPPP	chrom 6, unknown; ring finger protein 5	Human fetal brain cDNA 3'-end GEN-099F03.	1345	P	731	P	NC	-1,7	-0,4	738	P	NC	-1,8	-0,58	722	P	NC	-1,9	-0,63	663	P	D	-5,7	-9,71
RC_H96850_at	PPPPP	*oligosaccharyltransferase d89060 1p36.1 (also C-class)*	yw03b12.s1 Soares melanocyte 2NbHM Homo sapiens cDNA clone 251135 3' similar to contains Alu repetitive element;.	4876	P	3348	P	NC	-1,5	-0,45	4062	P	NC	-1,2	-0,13	3685	P	NC	-1,3	-0,27	2211	P	D	-6,2	-20,52
RC_AA127444_at	PPPPP	chrom 1 *no homology*	zn92e09.s1 Stratagene lung carcinoma 937218 Homo sapiens cDNA clone 565672 3' similar to TR:G563829 G563829 FATTY ACID TRANSPORT PROTEIN. ;.	626	P	353	P	NC	-1,8	-0,36	364	P	NC	-1,7	-0,33	427	P	NC	-1,5	-0,17	264	P	D	-6,6	-8,01

RC:AA424824_at	PPPPP	chrom 11; ac005233 PAC clone chrom 22	zr65e11.s 1 Soares NhHMPu S1 Homo sapiens cDNA clone 668300 3'.	710	P	178	P	NC	-2,2	-0,52	246	P	NC	-1,3	-0,07	144	P	N C	-2,1	-0,46	188	P	D	-8,95
																								8,4
AA405775_s_at	PPPPP	similar to CAA16821 (PID:g3255952)	zu57c10.r 1 Soares ovary tumor NbHOT Homo sapiens cDNA clone 742098 5'.	1047	P	692	P	NC	-1,5	-0,25	804	P	NC	-1,3	-0,11	791	P	N C	-1,3	-0,12	135	P	D	-4,92
																								6,1
AB classifiers																								
Gained in Duke AB and Inc; NC in other Dukes																								
Avg Diff in AB >= 300																								
RC_AA136269_at	APPAA	HSPC314, chrom 12	zk93b07.s 1 Soares pregnant uterus NbHPU Homo sapiens cDNA clone 490357 3'.	184	A	319	P	I	2,9	1,25	266	P	I	2,9	1,25	85	A	N C	1,8	0,28	154	A	N C	1,7 0,2
Lost in Duke AB and Dec; NC in other Dukes																								
Avg Diff in N > 300																								
RC_T40895_at	PAAPP	protein tyrosine phosphatase PTPCAAX1; protein tyrosine phosphatase hPRL-1N; iva-1, chrom 6	ya13f02.s1 Homo sapiens cDNA clone 61371 3'.	1154	P	244	A	D	-3,1	-1,64	41	A	D	-.. 12,7	-9,62	720	P	D	-1,6	-0,34	1079	P	N C	-0,01 1,1
RC:AA424400_at	PAAPP	chrom 14	zv82e05.s	344	P	123	A	D	-2,8	-0,91	120	A	D	-2,9	-0,97	180	P	N	-1,9	-0,35	166	P	D	-5,07

[illegible]			1 Soares total fetus Nb2HF8 9w Homo sapiens cDNA clone 760160 3'.															C						5,8	
PPPPP; Inc in Duke AB																									
Avg Diff AB >= 300; Fold change N to AB >= 5x																									
RC_N22015_at	PPPPP	unnamed protein product, chrom 17 ; BC class.	yw31h10.s 1 Homo sapiens cDNA clone 253891 3'.	638	P	3335	P	I	5,2	8,09	3608	P	I	5,3	8,6	2944	P	I	4,4	5,98	2893	P	I	4,5	6,17
RC_N50971_at	PPPPP	-	yy94b07.s 1 Homo sapiens cDNA clone 281173 3'.	129	P	1031	P	I	10	10,43	581	P	I	6,8	4,78	761	P	I	5,9	4,62	599	P	I	3,1	1,71
RC_AA194833_at	PPPPP	senescence-associated epithelial membrane protein; Claudin-1, chrom 3	zr31b04.s 1 Soares NhHMPu S1 Homo sapiens cDNA clone 664975 3'.	185	P	999	P	I	5,7	5,26	1316	P	I	7,6	8,35	328	P	I	3,7	1,78	1281	P	I	7,3	7,88
RC_AA393803_at	PPPPP	chrom 6	zv64c05.s 1 Soares total fetus Nb2HF8 9w Homo sapiens cDNA clone 758408 3'.	69	P	310	P	I	5,7	2,8	503	P	I	9,2	6,04	359	P	I	6,6	3,59	400	P	I	7,3	4,28

BC-classifiers																									
Gained in Duke BC and Inc; NC in other Dukes																									
Avg Diff in BC > 300																									
RC_AA148923_at	AAPPA	DEPP; decidual protein induced by progesterone; hypothetical protein, chrom 10	zl27g11.s1 Soares pregnant uterus NbHPU Homo sapiens cDNA clone 503204 3'.	233	A	212	A	NC	1,2	0,04	731	P	I	4	2,7	507	P	I	2,8	1,11	235	A	NC	1,2	0,02
Lost in Duke BC and Dec																									
Avg Diff in N > 300																									
RC_N91919_s_at	PPAAP	mineralocorticoid receptor; mineralocorticoid receptor (aldosterone receptor); chrom 4	zb48e05.s1 Soares fetal lung NbHL19W Homo sapiens cDNA clone 306848 3' similar to gb:M16801 MINERALOCORTICOID RECEPTOR (HUMAN);.	618	P	206	P	D	-2,4	-0,82	16	A	D	~-13,4	-7,34	39	A	D	~-12,0	-7,51	136	P	D	-3,7	-1,89
RC_T99196_s_at	PPAAP	chrom 5 *AF010235 Homo sapiens mRNA from chromosome 5q31-33 region*	ye62f11.s1 Homo sapiens cDNA clone 122349 3'.	529	P	274	P	D	-2,2	-0,67	143	A	D	-3,7	-1,94	197	A	D	-2,7	-1,04	221	P	D	-2,7	-1,13
RC_W72366_at	PPAAP	chrom 1 *clone RP11-404F10 on chromo-*	zd62f01.s1 Soares	323	P	183	P	NC	-1,8	-0,26	69	A	D	-4,7	-2,2	115	A	D	-2,8	-0,89	140	P	D	-6,4	-5,55

		some 1q23.1-24.1, CD48 hom	fetal heart NbHH19W Homo sapiens cDNA clone 345241 3' similar to gb:M81181 SODI-UM/POTASSIUM-TRANS-PORTING ATPASE BETA-2 (HU-MAN);contains Alu repetitive element;.																						
RC_AA255903_at	PPAAP	CD39L4; CD39-like 4; chrom 14	zs28d05.s1 NCI_CGAP_GCB1 Homo sapiens cDNA clone IMAGE:686505 3'.	840	P	260	P	NC	-3,4	-2,13	-36	A	D	~-25,4	-15,91	153	A	D	-5,7	-4,69	204	P	MD	-11,5	-15,73
PPPPP; Inc In Duke BC																									
Avg Diff N >= 300; Fold change N to BC >= 5x																									
J03464_s_at	PPPPP	collagen alpha-2 type I mRNA COL2A1	Human collagen alpha-2 type I mRNA, complete cds, clone pHCOL2A1	352	P	1494	P	I	4,2	4,09	3072	P	I	8,7	14,19	2027	P	I	5,8	7,31	2206	P	I	6,3	8,46

RC_N22015_at	PPPPP	unnamed protein product, chrom 17	yw31h10.s1 Homo sapiens cDNA clone 253891 3'.	638	P	3335	P	I	5,2	8,09	3608	P	I	5,3	8,6	2944	P	I	4,4	5,98	2893	P	I	4,5	6,17
RC_AA055896_at	PPPPP	chrom 9	zf20e03.s1 Soares fetal heart NbHH19W Homo sapiens cDNA clone 377500 3'.	590	P	1835	P	I	2,9	2,15	3885	P	I	6,4	11,26	2467	P	I	3,9	4,33	1553	P	MI	2,5	1,48
RC_AA121315_at	PPPPP	KIAA1077 protein; hypothetical protein, chrom 8	zk91g08.s1 Soares pregnant uterus NbHPU Homo sapiens cDNA clone 490238 3'.	429	P	1764	P	I	4,9	5,57	3493	P	I	9,8	16,81	1724	P	I	4,8	5,33	1220	P	NC	3,4	2,57
RC_AA122386_at	PPPPP	procollagen alpha 2(V); pro- alpha (V)collagen (AA 1099), chrom 2	zn36f10.s1 Stratagene endothelial cell 937223 Homo sapiens cDNA clone 549547 3'.	466	P	1403	P	I	3	2,15	2356	P	I	5,1	6,63	1725	P	I	3,7	3,51	1716	P	I	3,7	3,47
PPPPP; DEC in Duke BC																									
Avg Diff N >= 500; Fold change N to BC >= 5x																									
C02386_s_at	PPPPP		HUMGS0010652, Human Gene Signature,	3429	P	1667	P	D	-2,1	-1,35	469	P	D	-7,1	-11,91	640	P	D	-5,4	-8,65	969	P	D	-3,5	-4,58

			3'-directed cDNA sequence.																						
M12272_s_at	PPPPP	alcohol dehydrogena-se class I gamma subunit (ADH3)	Homo sapiens alcohol dehydro-genase class I gamma subunit (ADH3) mRNA, complete cds	3368	P	904	P	D	-3,4	-4,07	266	P	D	-11,6	-18,17	120	P	D	-25,7	-30	290	P	D	-10,6	-16,96
M12759_at	PPPPP	Ig J chain gene	Human Ig J chain gene	2628	P	686	P	D	-3,2	-3,14	239	P	D	-8,6	-11,42	107	P	D	-19,2	-20,94	190	P	D	-11,7	-15,55
M83670_s_at	PPPPP	carbonic anhydrase IV	Human carbonic anhydrase IV mRNA, complete cds	2138	P	432	P	D	-5,1	-6	249	P	D	-9,6	-12,13	342	P	D	-6,6	-8,32	249	P	D	-9,4	-11,84
N91087_at	PPPPP	dJ991C6.1 (novel protein similar to C. elegans F55A12.9 (Tr:P91086)); unna-med protein product; chrom 6	za18b11.r1 Soares fetal liver spleen 1NFLS Homo sapiens cDNA clone 292893 5'.	923	P	217	P	D	-4,4	-3,43	173	P	D	-5,3	-4,45	167	P	D	-5,5	-4,68	350	P	D	-2,6	-1,32
RC_H77597_f_at	PPPPP	unident. EST chrom /// AFFY = similar metal-lothionein	ys08a06.s1 Homo sapiens cDNA clone 214162 3' similar to gb:X64177 H.sapiens mRNA for	5411	P	1118	P	D	-4,4	-8,23	762	P	D	-7,1	-15,26	619	P	D	-8,7	-18,86	979	P	D	-15,4	-49,46

			metallothionein (HUMAN);.																						
RC_N23665_s_at	PPPPP	L11708 ESTRADIOL 17 BETA-DEHYDROGENASE 2, chrom 16	yw40b12.s1 Homo sapiens cDNA clone 254687 3' similar to gb:L11708 ESTRADIOL 17 BETA-DEHYDROGENASE 2 (HUMAN);.	1150	P	246	P	D	-4,1	-3,18	84	P	D	-12	-10,65	68	P	D	-14	-11,67	143	P	D	-22,4	-28,45
RC_N79237_at	PPPPP	unnamed protein product, hom to S49589 cortical granule lectin - African clawed frog ;.	za63a11.s1 Homo sapiens cDNA clone 297212 3' similar to PIR:S49589 S49589 cortical granule lectin - African clawed frog ;.	7342	P	2621	P	D	-2,8	-4,25	237	P	D	-29,6	-48,42	458	P	D	-15,7	-34,48	499	P	D	-40,1	-92,94
RC_N80129_f_at	PPPPP	MT-1l protein; metallothionein IX; metallothionein 1L, chrom 16 & chrom 1	za65a05.s1 Homo sapiens cDNA clone 297392 3' similar to gb:X76717 H.sapiens MT-1l	3623	P	1009	P	D	-3,6	-4,83	323	P	D	-11,2	-19,21	516	P	D	-24,5	-31,69	737	P	D	-14,8	-40,79

			mRNA. (HUMAN);.																						
RC_T24011_at	PPPPP	-	seq2167 Homo sapiens cDNA clone 3HFLSK20-53 3'.	900	P	317	P	D	-2,8	-1,53	177	P	D	-5,1	-4,15	157	P	D	-5,7	-4,85	371	P	D	-2,8	-1,54
RC_T90492_at	PPPPP	IGJ, P01591 IMMU-NOGLOBULIN J ;.chrom 4	ye15c08.s1 Homo sapiens cDNA clone 117806 3' similar to SP:IGJ_HUMAN P01591 IMMU-NOGLO-BULIN J ;.	4498	P	1829	P	D	-2,5	-2,47	468	P	D	-10,3	-20,59	321	P	D	-15	-27,44	623	P	D	-21,6	-57,11
RC_AA058357_s_at	PPPPP	singleton; no hom. , NONSPECIFIC CROSSREACTING ANTIGEN. ;.	zl67e01.s1 Stratagene colon (#937204) Homo sapiens cDNA clone 509688 3' similar to TR:G189087 G189087 NONSPECIFIC CROSS-REAC-TING ANTIGEN. ;.	5524	P	1757	P	D	-3,1	-4,66	232	P	D	-20,3	-32,79	460	P	D	-11,3	-23,01	1392	P	D	-3,7	-6,14
RC_AA133469_at	PPPPP	cytokeratin 20, chrom 17	zo13e11.s1 Strata-	3504	P	1231	P	D	-2,4	-1,87	453	P	D	-6,5	-10,1	375	P	D	-7,9	-12,5	1359	P	D	-2,6	-2,44

			gene colon (#937204) Homo sapiens cDNA clone 586796 3'.													8					2				
RC_AA151674_at	PPPPP	carbonic anhydrase XII precursor; chrom 15	zo29e02.s1 Stratagene colon (#937204) Homo sapiens cDNA clone 588314 3'.	1820	P	357	P	D	-5,1	-5,9	188	P	D	-8,8	-10,55	116	P	D	-13,7	-14,72	327	P	D	-5,6	-6,63
RC_AA405715_at	PPPPP	unknown	zu66d01.s1 Soares testis NHT Homo sapiens cDNA clone 742945 3'.	4188	P	1080	P	D	-3,9	-5,93	542	P	D	-7,7	-14,69	521	P	D	-8	-15,29	1120	P	D	-10,4	-32,45
RC_AA428410_at	PPPPP	KIAA0828 protein, chrom 7	zw57g04.s1 Soares total fetus Nb2HF8 9w Homo sapiens cDNA clone 774198 3' similar to contains Alu repetitive element;contains element PTR7 repetitive element ;.	964	P	277	P	D	-3	-1,72	141	P	D	-8,6	-6,99	53	P	D	-14,8	-10,91	79	P	D	-9,9	-8
RC_AA429253_at	PPPPP	KIAA0803 protein;	zv50c08.s	555	P	279	P	D	-2	-0,49	103	P	D	-5,4	-3,5	111	P	D	-5	-3,15	213	P	D	-	-8,35

		Centrosome- and Golgi-localized PKN-associated protein (CG-NAP); AKAP450, A-kinase anchoring protein AKAP350, chrom 7	1 Soares ovary tumor NbHOT Homo sapiens cDNA clone 757070 3'.																					7,3	
RC_AA487468_at	PPPPP	chrom 7	ab23b07.s 1 Strata-gene lung (#937210) Homo sapiens cDNA clone 841621 3'.	820	P	263	P	D	-2,6	-1,13	106	P	D	-6,5	-4,92	95	P	D	-7,2	-5,56	319	P	D	-2,6	-1,17
RC_AA621680_at	PPPPP	zinc finger transcription factor GKLF; EZF; hEZF; endothelial Kruppel-like zinc finger protein, transforming oncogene	af48e09.s 1 Soares total fetus Nb2HF8 9w Homo sapiens cDNA clone 1034920 3'.	1201	P	295	P	D	-4,1	-3,43	110	P	D	-10,9	-10,81	124	P	D	-9,7	-9,77	371	P	D	-3,2	-2,3
Y09616_at	PPPPP	putative carboxylesterase	H.sapiens mRNA for putative carboxylesterase	1776	P	605	P	D	-3	-2,48	299	P	D	-6,1	-7,47	260	P	D	-7	-8,77	333	P	D	-5,5	-6,52
AA171913_at	PPPPP	carbonic anhydrase XII (CA12) chrom 15	zo95d05.r 1 Strata-gene ovarian cancer (#937219) Homo sapiens cDNA clone 594633 5'.	1117	P	148	P	D	-7,2	-6,85	78	P	D	-13,6	-11,99	85	P	D	-51,6	-23,46	148	P	D	-7,2	-6,84

AA253330_s_at	PPPPP	hypothetical protein; unnamed protein product, chrom 15	zr72g02.r1 Soares NhHMPu S1 Homo sapiens cDNA clone 668978 5'.	2590	P	740	P	D	-3,5	-3,91	448	P	D	-6	-8,89	420	P	D	-6,4	-9,59	697	P	D	-3,7	-4,33

B. *Finding potential classifier genes for colorectal cancer (Dukes A, B, C & D) by sorting according to Pearson correlation coefficient*

Primary selection criteria for classifier genes:

1. All genes with a score of A (AbsCall) or NC (DiffCall) for all groups (N, A, B, C & D) were removed.
2. Genes with a fold change below 5 and a Sort Score below 0.5 were removed.
3. If DiffCall were NC for a gene in a particular experiment the FC were set to 1.

Secondary selection criteria for classifier genes:

Based on Pearson correlation coefficient (figure 1) genes similar to a predefined profile were selected.

$$r = \frac{n(\Sigma XY) - (\Sigma X)(\Sigma Y)}{\sqrt{\left[n\Sigma X^2 - (\Sigma X)^2\right]\left[n\Sigma Y^2 - (\Sigma Y)^2\right]}}$$

Figure 1: Pearson correlation coefficient (*r*)

Classifier genes for Dukes A, B, C and D:

Table III

A classifiers (Profile 1, 0, 0, 0), Pearson correlations approach

Hu6800	
D87444_at	Human mRNA for KIAA0255 "gene," complete cds
U18291_at	Human CDC16Hs "mRNA," complete cds
L76568_xpt3_f_at	S26 from Homo sapiens excision and cross link repair protein (ERCC4) "gene," complete genomic sequence. /gb=L76568 /ntype=DNA /annot=exon
U45328_s_at	"Human ubiquitin-conjugating enzyme (UBE2I) ""mRNA,"" complete cds"
Z14982_rna1_at	H.sapiens gene for major histocompatibility complex encoded proteasome subunit LMP7.
AD000092_cds7_s_at	RAD23A gene (human RAD23A homolog) extracted from Homo sapiens DNA from chromosome 19p13.2 cosmids "R31240," R30272 and R28549 containing the "EKLF," "GCDH," "CRTC," and RAD23A "genes," genomic sequence
D86973_at	Human mRNA for KIAA0219 "gene," partial cds
X81636_at	H.sapiens clathrin light chain a gene
M59916_at	Human acid sphingomyelinase (ASM) "mRNA," complete cds
X85781_s_at	"H.sapiens NOS2 ""gene,"" exon 27 /gb=X85781 /ntype=DNA /annot=exon"
M57731_s_at	"Human gro-beta ""mRNA,"" complete cds"
U49188_at	Human placenta (Diff33) "mRNA," complete cds
X53800_s_at	Human mRNA for macrophage inflammatory protein-2beta (MIP2beta)
U56816_at	Human kinase Myt1 (Myt1) "mRNA," complete cds.
HG1067-HT1067_r_at	Mucin (Gb:M22406)
EST:	
RC_F03077_f	Chromosome 17, clone hRPC.15

RC_AA599199	Alu seq
RC_AA207015	clone RP4-733M16 on chromosome 1p36.11-36.23
RC_AA234916	Chromosome 19 clone CTC-461H2
RC_N92239_a	Wnt inhibitory factor-1 (WIF-1), chromosome 12
RC_N93958_s	Phospholipase A2, group X (PLA2G10),
U95301_at	Phospholipase A2, group X (PLA2G10),
RC_AA426330	Chromosome 17, clone hRPC.1110_E_20
RC_AA024658	clone SCb-254N2 (UWGC:rg254N02) from 6p21
RC_H88540_a	heat shock protein 90, 1q21.2-q22

B classifiers (Profile 0, 1, 0, 0)

Hu6800:

U57316_at	Human GCN5 (hGCN5) "gene," complete cds
X66839_at	H.sapiens MaTu MN mRNA for p54/58N protein
J04599_at	Human hPGI mRNA encoding bone small proteoglycan I "(biglycan)," complete cds
X57579_s_at	H.sapiens activin beta-A subunit (exon 2)
J02874_at	Human adipocyte lipid-binding "protein," complete cds
M11749_at	Human Thy-1 glycoprotein "gene," complete cds
U06863_at	Human follistatin-related protein precursor "mRNA," complete cds
U51010_s_at	"Human nicotinamide N-methyltransferase ""gene,"" exon 1 and 5' flanking region. /gb=U51010 /ntype=DNA /annot=exon"
U08021_at	"Human nicotinamide N-methyltransferase (NNMT) ""mRNA,"" complete cds"
HG3044-HT3742_s_at	"""Fibronectin,"" Alt. Splice 1"
X02761_s_at	Human mRNA for fibronectin (FN precursor)
X02544_at	Human mRNA for alpha1-acid glycoprotein (orosomucoid)
M62505_at	Human C5a anaphylatoxin receptor "mRNA," complete cds
J05070_at	Human type IV collagenase "mRNA," complete cds
U16306_at	Human chondroitin sulfate proteoglycan versican V0 splice-variant precursor peptide "mRNA," complete cds
M14218_at	Human argininosuccinate lyase "mRNA," complete cds
L77567_s_at	"Homo sapiens mitochondrial citrate transport protein (CTP) ""mRNA,"" 3' end"
M63391_rna1_at	Human desmin gene, complete cds.
D13643_at	Human mRNA for KIAA0018 "gene," complete cds
D79985_at	Human mRNA for KIAA0163 "gene," complete cds

EST:

M63262_at	5-lipoxygenase activating protein (FLAP), 13q12
R67290_at	Interleukine 14
N36619_at	
L19161_at	Translation initiation factor 2, subunit 3", Xp22.2-22.1
RC_AA496035	Chromosome 1? (TIGR)
L29217_s_at	CDC-like kinase 3 (CLK3), 15q24
RC_W73194_a	Dermatoponin, 1q12-q23
RC_N69507_a	Hypothetical protein PRO1847 (Alu according to TIGR)
RC_H15814_s	adipose most abundant gene transcript 1
M84526_at	D component of complement (adipsin)

C classifiers (Profile 0, 0, 1, 0)

Hu6800:

M20681_at	Human glucose transporter-like protein-III "(GLUT3)," complete cds
D50914_at	Human mRNA for KIAA0124 "gene," partial cds
L37362_at	Homo sapiens (clone d2-115) kappa opioid receptor (OPRK1) "mRNA," complete cds
X66114_rna1_at	H.sapiens gene for 2-oxoglutarate carrier protein.
M32053_at	Human H19 RNA "gene," complete cds (spliced in silico)

Y00787_s_at	Human mRNA for MDNCF (monocyte-derived neutrophil chemotactic factor)
U64444_at	Human ubiquitin fusion-degradation protein (UFD1L) "mRNA," complete cds
X95325_s_at	H.sapiens mRNA for DNA binding protein A variant
X02419_rna1_s_at	H.sapiens uPA gene
X57522_at	H.sapiens RING4 cDNA
AB001325_at	Human AQP3 gene for aquaporine 3 (water "channel)," partail cds
AB002315_at	Human mRNA for KIAA0317 "gene," complete cds. /gb=AB002315 /ntype=RNA
L12760_s_at	"Human phosphoenolpyruvate carboxykinase (PCK1) ""gene,"" complete cds with repeats"
M80899_at	Human novel protein AHNAK "mRNA," partial sequence

EST:

RC_AA122350	Chromosome 8
AA374109_at	spondin 2, extracellular matrix protein, chromosome 4
RC_AA621755	Transcription factor Dp-2, 3q23
RC_AA442069	sodium channel 2, 12q12
RC_T40767_a	Chromosome 19
RC_AA488655	Mus?
RC_AA398908	
RC_AA447764	Hypothetical protein, chromosome 4
RC_N69136_a	

D classifiers (Profile 0, 0, 0, 1)

X17644_s_at	Human GST1-Hs mRNA for GTP-binding protein
Y12812_at	H.sapiens RFXAP mRNA
X60486_at	H.sapiens H4/g gene for H4 histone
X52221_at	H.sapiens ERCC2 "gene," exons 1 & 2 (partial)
L06175_at	Homo Sapiens P5-1 "mRNA," complete cds
Z48481_at	H.sapiens mRNA for membrane-type matrix metalloproteinase 1
X54232_at	Human mRNA for heparan sulfate proteaglycan (glypican)
L08010_at	"Homo sapiens reg gene ""homologue,"" complete cds"
L27706_at	Human chaperonin protein (Tcp20) gene complete cds
L15533_rna1_at	Homo sapiens pancreatits-associated protein (PAP) gene, complete cds.
X51408_at	Human mRNA for n-chimaerin
K02765_at	Human complement component C3 "mRNA," alpha and beta "subunits," complete cds
U38904_at	Human zinc finger protein C2H2-25 "mRNA," complete cds

EST:

RC_AA121433	Axin, chromosome 16
RC_N91920_a	RB protein binding protein, chromosome 16
RC_AA621601	GTP-binding protein Rab36, chromosome 17
RC_AA454020	NADPH quinone oxidoreductase homolog; p53 induced, chromosome 2
RC_Z39652_a	APM-1 gene, chromosome 18

Conclusion.

As can be seen from these tables we have identified a number of genes and EST's, based on two different approaches, that we believe are either of importance for initiating and developing colorectal cancer, or can be used to classify the disease. These genes and EST's are subdivided into potential tumor suppressors that have a reduced level during progression of the disease – or that even completely lose their expression; potential oncogenes that increase their level during disease progression , or even are gained de novo, not being expressed at early stages or

in normal mucosa; and finally classifiers of the disease that can be used to identify the different Dukes stages , e.g. being only expressed at a certain stage.

Claims:

1. A method of determining the presence or absence of a biological condition in animal tissue

comprising collecting a sample comprising cells from the tissue and/or expression products from the cells,

assaying a first expression level of at least one gene from a first gene group, wherein the gene from the first gene group is selected from genes expressed in normal tissue cells in an amount higher than expression in biological condition cells, and/or

assaying a second expression level of at least one gene from a second gene group, wherein the second gene group is selected from genes expressed in a normal tissue cells in an amount lower than expression in biological condition cells,

correlating the first expression level to a standard expression level for normal tissue, and/or the second expression level to a standard expression level for biological condition cells to determine the presence or absence of a biological condition in the animal tissue.

2. The method of claim 1, wherein the animal tissue is selected from epithelial tissue.

3. The method of claim 2, wherein the animal tissue is selected from epithelial tissue in the gastro-intestinal tract.

4. The method of claim 3, wherein the animal tissue is selected from epithelial tissue in colon and/or rectum.

5. The method according to claim 4, wherein the animal tissue is mucosa.

6. The method of any of the preceding claims, wherein the biological condition is an adenocarcinoma, a carcinoma, a teratoma, a sarcoma, and/or a lymphoma.

7. The method of any of the preceding claims, wherein the sample is a biopsy of the tissue.

8. The method according to any of the preceding claim 1-6, wherein the sample is a cell suspension made from the tissue.

9. The method according to any of the preceding claims, wherein the sample comprises substantially only cells from said tissue.

10. The method according to claim 9, wherein the sample comprises substantially only cells from mucosa.

11. The method according to any of the claims 3-10, wherein the gene from the first gene group is selected individually from genes comprising a sequence as identified below

RC_H04768_at	chrom 15 no homology
RC_Z39652_at	Y14593 APM-1 gene adipocyte-specific secretory protein; chrom 1q21.3-q23
RC_H30270_at	chrom 18 PAAAA in colon & bladder no homology
RC_T47089_s_at	tenascin-X; tenascin-X precursor; unidentified protein
RC_W31906_at	secretagogin; dJ501N12.8 (putative protein) chrom 6
RC_AA279803_at	chrom 2 no homology
RC_R01646_at	chrom 13q32.1-33.3 ; AL159152 ; homology to mouse Pcbp1 - poly(rC)-binding protein 1
RC_AA099820_at	BAC clone AC016778
AA319615_at	secretory carrier membrane protein; secretory carrier membrane protein 2; chrom 15
H07011_at	tetraspan NET-6 mRNA; transmembrane 4 superfamily; chrom 7
RC_T68873_f_at	
RC_T40995_f_at	
RC_H81070_f_at	
RC_N30796_at	
RC_W37778_f_at	
RC_R70212_s_at	
RC_AA426330_at	

RC_N33927_s_at
RC_T90190_s_at
RC_AA447145_at
RC_H75860_at
RC_T71132_s_at

wherein the notation refers to Accession No. in the database UniGene (Build 18).

12. The method according to claim 11, wherein the gene from the first gene group is selected individually from genes comprising a sequence as identified below

RC_H04768_at	chrom 15 no homology
RC_Z39652_at	Y14593 APM-1 gene adipocyte-specific secretory protein; chrom 1q21.3-q23
RC_H30270_at	chrom 18 PAAAA in colon & bladder no homology
RC_T47089_s_at	tenascin-X; tenascin-X precursor; unidentified protein
RC_W31906_at	secretagogin; dJ501N12.8 (putative protein) chrom 6
RC_AA279803_at	chrom 2 no homology
RC_R01646_at	chrom 13q32.1-33.3 ; AL159152 ; homology to mouse Pcbp1 - poly(rC)-binding protein 1
RC_AA099820_at	BAC clone AC016778
AA319615_at	secretory carrier membrane protein; secretory carrier membrane protein 2; chrom 15
H07011_at	tetraspan NET-6 mRNA; transmembrane 4 superfamily; chrom 7

wherein the notation refers to Accession No. in the database UniGene (Build 18).

13. The method according to claim 12, wherein the gene from the first gene group is selected individually from genes comprising a sequence as identified below

RC_H04768_at	chrom 15 no homology
RC_Z39652_at	Y14593 APM-1 gene adipocyte-specific secretory protein; chrom 1q21.3-q23
RC_H30270_at	chrom 18 PAAAA in colon & bladder no homology
RC_T47089_s_at	tenascin-X; tenascin-X precursor; unidentified protein

RC_W31906_at	secretagogin; dJ501N12.8 (putative protein) chrom 6
RC_AA279803_at	chrom 2 no homology
RC_R01646_at	chrom 13q32.1-33.3 ; AL159152 ; homology to mouse Pcbp1 - poly(rC)-binding protein 1
AA319615_at	secretory carrier membrane protein; secretory carrier membrane protein 2; chrom 15

wherein the notation refers to Accession No. in the database UniGene (Build 18).

14. The method according to claim 13, wherein the gene from the first gene group is selected individually from genes comprising a sequence as identified below

RC_T47089_s_at	tenascin-X; tenascin-X precursor; unidentified protein
RC_W31906_at	secretagogin; dJ501N12.8 (putative protein) chrom 6
RC_AA279803_at	chrom 2 no homology
AA319615_at	secretory carrier membrane protein; secretory carrier membrane protein 2; chrom 15

wherein the notation refers to Accession No. in the database UniGene (Build 18)

15. The method according to any of claims 3-14, wherein the second gene group are selected individually from genes comprising a sequence as identified below

RC_AA609013_s_at	microsomal dipeptidase (also on 6.8k); chrom 16
RC_AA232508_at	CGI-89 protein; unnamed protein product; hypothetical protein
RC_AA428964_at	serine protease-like protease; serine protease homolog=NES1; normal epithelial cell-specific 1
RC_T52813_s_at	dJ28O10.2 (G0S2 (PUTATIVE LYMPHOCYTE G0/G1 SWITCH PROTEIN 2; chrom 1
RC_AA075642_at	gp-340 variant protein; DMBT1/8kb.2 protein
RC_AA007218_at	chrom 13 no homology
RC_N33920_at	ubiquitin-like protein FAT10; diubiquitin; dJ271M21.6 (Diubiquitin); chrom 6
RC_N71781_at	KIAA1199 protein, chrom 15
RC_R67275_s_at	alpha-1 (type XI) collagen precursor; collagen, type XI, alpha 1; collagen type XI alp

	ha-1 isoform A; chrom 1
RC_W80763_at	hypothetical protein; chrom 17
RC_AA443793_at	chrom 7p22 AC006028 BAC clone
RC_AA034499_s_at	ZNF198 protein; zinc finger protein; FIM protein; Cys-rich protein; zinc finger protein 198; chrom 13
RC_AA035482_at	chrom 5; AK022505 clone; CalcineurinB (weakly similar)
RC_AA024482_at	hypothetical protein; unnamed protein product; chrom 17
RC_H93021_at	chrom 2 ; XM_004890 peptidylprolyl isomerase A (cyclophilin A)
RC_AA427737_at	no homology
RC_AA417078_at	chrom 7q31; AF017104 clone
M29873_s_at	cytochrome P450-IIB (hIIB3) ; 19q13.1-q13.2
RC_H27498_f_at	
RC_T92363_s_at	
RC_N89910_at	
RC_W60516_at	
RC_AA219699_at	
RC_AA449450_at	

wherein the notation refers to Accession No. in the database UniGene (Build 18).

16. The method according to any of claims 3-15, wherein the second gene group are selected individually from genes comprising a sequence as identified below

RC_AA609013_s_at	microsomal dipeptidase (also on 6.8k); chrom 16
RC_AA232508_at	CGI-89 protein; unnamed protein product; hypothetical protein
RC_AA428964_at	serine protease-like protease; serine protease homolog=NES1; normal epithelial cell-specific 1
RC_T52813_s_at	dJ28O10.2 (G0S2 (PUTATIVE LYMPHOCYTE G0/G1 SWITCH PROTEIN 2; chrom 1
RC_AA075642_at	gp-340 variant protein; DMBT1/8kb.2 protein
RC_AA007218_at	chrom 13 no homology
RC_N33920_at	ubiquitin-like protein FAT10; diubiquitin; dJ271M21.6 (Diubiquitin); chrom 6
RC_N71781_at	KIAA1199 protein, chrom 15
RC_R67275_s_at	alpha-1 (type XI) collagen precursor; collagen, type XI, alpha 1; collagen type XI alpha-1 isoform A; chrom 1

RC_W80763_at	hypothetical protein; chrom 17
RC_AA443793_at	chrom 7p22 AC006028 BAC clone
RC_AA034499_s_at	ZNF198 protein; zinc finger protein; FIM protein; Cys-rich protein; zinc finger protein 198; chrom 13
RC_AA035482_at	chrom 5; AK022505 clone; CalcineurinB (weakly similar)
RC_AA024482_at	hypothetical protein; unnamed protein product; chrom 17
RC_H93021_at	chrom 2 ; XM_004890 peptidylprolyl isomerase A (cyclophilin A)
RC_AA427737_at	no homology
RC_AA417078_at	chrom 7q31; AF017104 clone
M29873_s_at	cytochrome P450-IIB (hIIB3) ; 19q13.1-q13.2

wherein the notation refers to Accession No. in the database UniGene (Build 18).

17. The method according to any of claims 3-14, wherein the second gene group are selected individually from genes comprising a sequence as identified below

RC_AA609013_s_at	microsomal dipeptidase (also on 6.8k); chrom 16
RC_AA232508_at	CGI-89 protein; unnamed protein product; hypothetical protein
RC_AA428964_at	serine protease-like protease; serine protease homolog=NES1; normal epithelial cell-specific 1
RC_AA075642_at	gp-340 variant protein; DMBT1/8kb.2 protein
RC_AA007218_at	chrom 13 no homology
RC_N33920_at	ubiquitin-like protein FAT10; diubiquitin; dJ271M21.6 (Diubiquitin); chrom 6
RC_N71781_at	KIAA1199 protein, chrom 15
RC_R67275_s_at	alpha-1 (type XI) collagen precursor; collagen, type XI, alpha 1; collagen type XI alpha-1 isoform A; chrom 1
RC_W80763_at	hypothetical protein; chrom 17
RC_AA034499_s_at	ZNF198 protein; zinc finger protein; FIM protein; Cys-rich protein; zinc finger protein 198; chrom 13
RC_AA035482_at	chrom 5; AK022505 clone; CalcineurinB (weakly similar)
RC_AA024482_at	hypothetical protein; unnamed protein product; chrom 17
RC_H93021_at	chrom 2 ; XM_004890 peptidylprolyl isomerase A (cyclophilin A)
RC_AA427737_at	no homology

RC_AA417078_at	chrom 7q31; AF017104 clone
M29873_s_at	cytochrome P450-IIB (hIIB3) ; 19q13.1-q13.2

wherein the notation refers to Accession No. in the database UniGene (Build 18).

18. The method according to any of claims 3-17, wherein the second gene group comprises a sequence as identified below

RC_W80763_at		hypothetical protein; chrom 17

wherein the notation refers to Accession No. in the database UniGene (Build 18).

19. The method according to any of the preceding claims, wherein the expression level of at least two genes from the first gene group are determined.

20. The method according to any of the preceding claims, wherein the expression level of at least two genes from the second gene group are determined.

21. The method according to any of the preceding claims, further comprising the steps of determining the stage of a biological condition in the animal tissue, comprising assaying a third expression level of at least one gene from a third gene group, wherein a gene from said second gene group, in one stage, is expressed differently from a gene from said third gene group.

22. The method according to any of the preceding claims, wherein the difference in expression level of a gene from one group to the expression level of a gene from another group is at least two-fold.

23. The method according to any of the preceding claims, wherein the difference in expression level of a gene from one group to the expression level of a gene from another group is at least three-fold.

24. The method according to any of the preceding claims, wherein the expression level is determined by determining the mRNA of the cells.

25. The method according to any of the claims 1-23, wherein the expression level is determined by determining expression products, such as peptides, in the cells.

26. The method according to claim 25, wherein the expression level is determined by determining expression products, such as peptides, in the body fluids, such as blood, serum, plasma, faeces, mucus, sputum, cerebrospinal fluid, and/or urine.

27. A method of determining the stage of a biological condition in animal tissue,

comprising collecting a sample comprising cells from the tissue,

assaying the expression of at least a first stage gene from a first stage gene group and/or at least a second stage gene from a second stage gene group, wherein at least one of said genes is expressed in said first stage of the condition in a higher amount than in said second stage, and the other gene is a expressed in said first stage of the condition in a lower amount than in said second stage of the condition,

correlating the expression level of the assessed genes to a standard level of expression determining the stage of the condition.

28. The method according to claim 27, wherein the tissue is selected from the epithelial tissue in colon or rectum.

29. The method according to any of the preceding claims 27-28, wherein the difference in expression levels between a gene from one group to a gene from another group is at least one-fold.

30. The method according to any of the preceding claims 27-29, wherein the difference in expression levels between a gene from one group to a gene from another group is at least two-fold.

31. The method according to claim 27, wherein the stage is selected from colon cancer stages Dukes A, Dukes B, Dukes C, and Dukes D.

32. The method according to claim 31, comprising assaying at least the expression of Dukes A stage gene from a Dukes A stage gene group, at least one Dukes B stage gene from a Dukes B stage gene group, at least the expression of Dukes C stage gene from a Dukes C stage gene group, and at least one Dukes D stage gene from a Dukes D stage gene group, wherein at least one gene from each gene group is expressed in a significantly different amount in that stage than in one of the other stages.

33. The method according to claim 32, wherein at least one gene from each gene group is expressed in a significantly higher amount in that stage than in one of the other stages.

34. The method according to claim 33, wherein a Dukes A stage gene is selected individually from any gene comprising a sequence as identified below

RC_AA599199_at	ALU seq.
RC_R12694_at	unnamed protein product BAA91641, chrom 10
RC_H91325_s_at	aldolase B; aldolase B (aa 1-364); chrom 9
RC_N51709_at	chrom X
RC_N72610_at	-
RC_N69263_at	chrom 10; AK026414 clone (only 108 nt hom)
RC_T15817_f_at	iNOS, inducible nitric oxide synthase

wherein the notation refers to Accession No. in the database UniGene (Build 18).

35. The method according to claim 33, wherein a Dukes B stage gene is selected individually from any gene comprising a sequence as identified below

RC_T67463_s_at	cathepsin O2; X; K
RC_W94688_at	perilipin
RC_AA126743_at	Z97200 PAC chrom 1q24;

		PMX1 homeobox gene
RC_AA236547_at		no homology
RC_AA255567_at		angiopoietin-related protein-2; angiopoietin-like 2
RC_AA421256_at		-
RC_AA386386_s_at	PPPPP	-
RC_AA452549_at	PPPPP	PRO1659; hypothetical protein chrom 11

wherein the notation refers to Accession No. in the database UniGene (Build 18).

36. The method according to claim 33, wherein a Dukes C stage gene is selected individually from any gene comprising a sequence as identified below

RC_D45556_at		chrom 15; AL390085 clone
RC_W86214_at		
RC_AA039439_s_at		novel gene KIAA0134 protein 19q13.3
RC_AA128935_at		
RC_AA134158_s_at		class I homeodomain; homeobox protein, chrom 7
RC_AA232646_at		chrom 17, AF266756 sphingosine kinase (SPHK1
RC_AA401184_at		no homology
RC_AA436840_at		
RC_AA488655_at		
RC_AA181902_at	PPPPP	AC007201 on chrom 19 (only 80nt hom)

wherein the notation refers to Accession No. in the database UniGene (Build 18).

37. The method according to claim 33, wherein a Dukes D stage gene is selected individually from any gene comprising a sequence as identified below

RC_N91920_at	AAAAP	chrom 16p12-p11.2 ; XN_007994 retinoblastoma binding protein
RC_AA621601_at	AAAAP	chrom 17 XM_009868 RAB36 ARS oncogene family

wherein the notation refers to Accession No. in the database UniGene (Build 18).

38. The method according to claim 32, wherein at least one gene from each gene group is expressed in a significantly lower amount in that stage than in one of the other stages.

39. The method according to claim 38, wherein a Dukes A stage gene is selected individually from any gene comprising a sequence as identified below

RC_N32411_f_at	PAPPP	Myc-associated zinc-finger protein of human islet; chrom 16
RC_AA243858_at	PAPPP	KIAA0882 protein
RC_AA486283_at	PAPPP	ras-like protein; ras-related C3 botulinum toxin substrate; dJ20J23
RC_AA490930_at	PAPPP	chrom 18; KIAA1468 protein
RC_H54088_s_at	PPPPP	ribosomal protein L41
RC_H59052_f_at	PPPPP	fungal sterol-C5-desaturase homolog; ORF; thymosin beta-4
RC_R49198_s_at	PPPPP	-
RC_T73572_f_at	PPPPP	ferritin L-chain; L apoferritin
RC_AA477483_at	PPPPP	no matching est

wherein the notation refers to Accession No. in the database UniGene (Build 18).

40. The method according to claim 38, wherein a Dukes B stage gene is selected individually from any gene comprising a sequence as identified below

RC_D59847_at	PPAPP	proSAAS; granin-like neuroendocrine peptide precursor
RC_F05038_at	PPAPP	polyamine modulated factor-1; polyamine modulated factor 1
RC_N41059_at	PPAPP	chrom 3

RC_T23460_at	PPAPP	chrom 3; IFNAR2 21q22.11
RC_W42789_at	PPAPP	chrom 8 AF268037 C8ORF4 protein (C8ORF4) chrom 8 ORF
RC_AA460017_i_at	PPAPP	BAC clone chrom 16
RC_AA482127_at	PPAPP	KIAA1142 protein
RC_AA504806_at	PPAPP	chrom 2 AF052107 clone 23620 mRNA sequence
RC_T90037_at	PPPPP	unnamed protein product, chrom 4
RC_AA432130_at	PPPPP	KIAA0867 protein, chrom 12

wherein the notation refers to Accession No. in the database UniGene (Build 18).

41. The method according to claim 38, wherein a Dukes C stage gene is selected individually from any gene comprising a sequence as identified below

RC_N30231_at	PPPAP	Lsm4 protein; U6 snRNA-associated Sm-like protein LSm4; glycine-rich protein
RC_W73790_f_at	PPPAP	immunoglobulin-related protein 14.1; lambda L-chain C region; omega protein, chrom 22
RC_AA412184_at	PPPAP	chrom 1p36; d89060 dolichyl-diphosphooligosaccharide-protein glycosyltransferase
RC_AA521303_at	PPPAP	methionine adenosyltransferase regulatory beta subunit; dTDP-4-keto-6-deoxy-D-glucose 4-reductase, chrom 5
RC_AA461174_at	PPPPP	8p21.3-p22 AB020860 anti-oncogene
AA393432_s_at	PPPPP	chrom 2, Unknown; unnamed protein product A-AD20029

wherein the notation refers to Accession No. in the database UniGene (Build 18).

42. The method according to claim 38, wherein a Dukes D stage gene is selected individually from any gene comprising a sequence as identified below

RC_R72886_s_at	PPPPA	KIAA0422; adenylyl cyclase type VI, chrom 12
RC_AA026030_at	PPPPA	chrom 1
RC_Z39006_at	PPPPA	hypothetical protein, chrom 17
RC_AA435908_at	PPPPA	chrom 19; ac011491 clone and 20 nt hom. RAB2, RAS oncogene family
RC_AA057829_s_at	PPPPA	growth-arrest-specific protein; growth arrest-specific 6; AXL stimulatory factor, chrom 13
RC_R72087_at	PPPPA	chrom 5 EST; hom to chrom 20 AL356652 clone
RC_H04242_at	PPPPA	ras related protein Rab5b; RAB5B, member RAS oncogene family
RC_R97304_f_at	PPPPA	HLA-drb5; cell surface glycoprotein; MHC HLA-DR-beta chain precursor chrom 6
RC_N48609_at	PPPPA	chrom 11; AC004584 chrom 17
RC_W86850_f_at	PPPPA	chrom 22 ? X96924 mitochondrial citrate tranbsport region
RC_AA130603_at	PPPPA	ak024908 clone
RC_AA479610_at	PPPPA	singleton ak025344 clone
RC_AA490593_i_at	PPPPA	chrom 17 ? Synaptobrevin2 (VAMP2) AF135372
RC_AA054321_s_at	PPPPA	6p21 HLA class i region; AC004202 clone
RC_D60328_at	PPPPP	chrom 6, unknown; ring finger protein 5
RC_H96850_at	PPPPP	oligosaccharyltransferase d89060 1p36.1 (also C-class)
RC_AA127444_at	PPPPP	chrom 1 no homology
RC_AA242824_at	PPPPP	chrom 11; ac005233 PAC clone chrom 22
AA405775_s_at	PPPPP	similar to CAA16821 (PID:g3255952)

wherein the notation refers to Accession No. in the database UniGene (Build 18).

43. A method of determining an expression pattern of a colon cell sample, comprising:

collecting sample comprising colon and/or rectum cells and/or expression products from colon and/or rectum cells,

determining the expression level of two or more genes in the sample, wherein at least one gene belongs to a first group of genes, said gene from the first gene group being expressed in a higher amount in normal tissue than in biological condition cells, and wherein at least one other gene belongs to a second group of genes, said gene from the second gene group being expressed in a lower amount in normal tissue than in biological condition cells, and the difference between the expression level of the first gene group in normal cells and biological condition cells being at least two-fold, obtaining an expression pattern of the colon and/or rectum cell sample.

44. The method of claim 43, wherein the two or more genes exclude genes which are expressed in the submucosal, muscle, or connective tissue, whereby a pattern of expression is formed for the sample which is independent of the proportion of submucosal, muscle, or connective tissue cells in the sample.

45. The method of claim 44, comprising determining the expression level of one or more genes in the sample comprising predominantly submucosal, muscle, and connective tissue cells, obtaining a second pattern, subtracting said second pattern from the expression pattern of the colon and/or rectum cell sample, forming a third pattern of expression, said third pattern of expression reflecting expression of the colorectal mucosa or colorectal cancer cells independent of the proportion of submucosal, muscle, and connective tissue cells present in the sample.

46. The method of any of the preceding claims 43-45, wherein the sample is a biopsy of the tissue.

47. The method according to any of the preceding claim 43-46, wherein the sample is a cell suspension.

48. The method according to any of the preceding claims 43-47, wherein the sample comprises substantially only cells from said tissue.

49. The method according to claim 48, wherein the sample comprises substantially only cells from mucosa.

50. The method according to any of the claims 43-47, wherein the gene from the first gene group is selected individually from

RC_H04768_at	chrom 15 no homology
RC_Z39652_at	Y14593 APM-1 gene adipocyte-specific secretory protein; chrom 1q21.3-q23
RC_H30270_at	chrom 18 PAAAA in colon & bladder no homology
RC_T47089_s_at	tenascin-X; tenascin-X precursor; unidentified protein
RC_W31906_at	secretagogin; dJ501N12.8 (putative protein) chrom 6
RC_AA279803_at	chrom 2 no homology
RC_R01646_at	chrom 13q32.1-33.3 ; AL159152 ; homology to mouse Pcbp1 - poly(rC)-binding protein 1
RC_AA099820_at	BAC clone AC016778
AA319615_at	secretory carrier membrane protein; secretory carrier membrane protein 2; chrom 15
H07011_at	tetraspan NET-6 mRNA; transmembrane 4 superfamily; chrom 7
RC_T68873_f_at	
RC_T40995_f_at	
RC_H81070_f_at	
RC_N30796_at	
RC_W37778_f_at	
RC_R70212_s_at	
RC_AA426330_at	
RC_N33927_s_at	
RC_T90190_s_at	
RC_AA447145_at	
RC_H75860_at	
RC_T71132_s_at	

wherein the notation refers to Accession No. in the database UniGene (Build 18).

51. The method according to claim 50, wherein the gene from the first gene group is selected individually from genes comprising a sequence as identified below

RC_H04768_at	chrom 15 no homology
RC_Z39652_at	Y14593 APM-1 gene adipocyte-specific secretory protein; chrom 1q21.3-q23
RC_H30270_at	chrom 18 PAAAA in colon & bladder no homology
RC_T47089_s_at	tenascin-X; tenascin-X precursor; unidentified protein
RC_W31906_at	secretagogin; dJ501N12.8 (putative protein) chrom 6
RC_AA279803_at	chrom 2 no homology
RC_R01646_at	chrom 13q32.1-33.3 ; AL159152 ; homology to mouse Pcbp1 - poly(rC)-binding protein 1
RC_AA099820_at	BAC clone AC016778
AA319615_at	secretory carrier membrane protein; secretory carrier membrane protein 2; chrom 15
H07011_at	tetraspan NET-6 mRNA; transmembrane 4 superfamily; chrom 7

wherein the notation refers to Accession No. in the database UniGene (Build 18).

52. The method according to claim 51, wherein the gene from the first gene group is selected individually from genes comprising a sequence as identified below

RC_H04768_at	chrom 15 no homology
RC_Z39652_at	Y14593 APM-1 gene adipocyte-specific secretory protein; chrom 1q21.3-q23
RC_H30270_at	chrom 18 PAAAA in colon & bladder no homology
RC_T47089_s_at	tenascin-X; tenascin-X precursor; unidentified protein
RC_W31906_at	secretagogin; dJ501N12.8 (putative protein) chrom 6
RC_AA279803_at	chrom 2 no homology
RC_R01646_at	chrom 13q32.1-33.3 ; AL159152 ; homology to mouse Pcbp1 - poly(rC)-binding protein 1
AA319615_at	secretory carrier membrane protein; secre

	tory carrier membrane protein 2; chrom 15

wherein the notation refers to Accession No. in the database UniGene (Build 18).

53. The method according to claim 52, wherein the gene from the first gene group is selected individually from genes comprising a sequence as identified below

RC_T47089_s_at	tenascin-X; tenascin-X precursor; unidentified protein
RC_W31906_at	secretagogin; dJ501N12.8 (putative protein) chrom 6
RC_AA279803_at	chrom 2 no homology
AA319615_at	secretory carrier membrane protein; secretory carrier membrane protein 2; chrom 15

wherein the notation refers to Accession No. in the database UniGene (Build 18).

54. The method according to any of claims 3-14, wherein the second gene group are selected individually from genes comprising a sequence as identified below

RC_AA609013_s_at	microsomal dipeptidase (also on 6.8k); chrom 16
RC_AA232508_at	CGI-89 protein; unnamed protein product; hypothetical protein
RC_AA428964_at	serine protease-like protease; serine protease homolog=NES1; normal epithelial cell-specific 1
RC_T52813_s_at	dJ28O10.2 (G0S2 (PUTATIVE LYMPHOCYTE G0/G1 SWITCH PROTEIN 2; chrom 1
RC_AA075642_at	gp-340 variant protein; DMBT1/8kb.2 protein
RC_AA007218_at	chrom 13 no homology
RC_N33920_at	ubiquitin-like protein FAT10; diubiquitin; dJ271M21.6 (Diubiquitin); chrom 6
RC_N71781_at	KIAA1199 protein, chrom 15
RC_R67275_s_at	alpha-1 (type XI) collagen precursor; collagen, type XI, alpha 1; collagen type XI alpha-1 isoform A; chrom 1
RC_W80763_at	hypothetical protein; chrom 17
RC_AA443793_at	chrom 7p22 AC006028 BAC clone
RC_AA034499_s_at	ZNF198 protein; zinc finger protein; FIM protein; Cys-rich protein; zinc finger protein

	198; chrom 13
RC_AA035482_at	chrom 5; AK022505 clone; CalcineurinB (weakly similar)
RC_AA024482_at	hypothetical protein; unnamed protein product; chrom 17
RC_H93021_at	chrom 2 ; XM_004890 peptidylprolyl isomerase A (cyclophilin A)
RC_AA427737_at	no homology
RC_AA417078_at	chrom 7q31; AF017104 clone
M29873_s_at	cytochrome P450-IIB (hIIB3) ; 19q13.1-q13.2
RC_H27498_f_at	
RC_T92363_s_at	
RC_N89910_at	
RC_W60516_at	
RC_AA219699_at	
RC_AA449450_at	

wherein the notation refers to Accession No. in the database UniGene (Build 18).

55. The method according to any of claims 43-49, wherein the second gene group are selected individually from genes comprising a sequence as identified below

RC_AA609013_s_at	microsomal dipeptidase (also on 6.8k); chrom 16
RC_AA232508_at	CGI-89 protein; unnamed protein product; hypothetical protein
RC_AA428964_at	serine protease-like protease; serine protease homolog=NES1; normal epithelial cell-specific 1
RC_T52813_s_at	dJ28O10.2 (G0S2 (PUTATIVE LYMPHOCYTE G0/G1 SWITCH PROTEIN 2; chrom 1
RC_AA075642_at	gp-340 variant protein; DMBT1/8kb.2 protein
RC_AA007218_at	chrom 13 no homology
RC_N33920_at	ubiquitin-like protein FAT10; diubiquitin; dJ271M21.6 (Diubiquitin); chrom 6
RC_N71781_at	KIAA1199 protein, chrom 15
RC_R67275_s_at	alpha-1 (type XI) collagen precursor; collagen, type XI, alpha 1; collagen type XI alpha-1 isoform A; chrom 1
RC_W80763_at	hypothetical protein; chrom 17
RC_AA443793_at	chrom 7p22 AC006028 BAC clone
RC_AA034499_s_at	ZNF198 protein; zinc finger protein; FIM protein; Cys-rich protein; zinc finger protein 198; chrom 13

RC_AA035482_at	chrom 5; AK022505 clone; CalcineurinB (weakly similar)
RC_AA024482_at	hypothetical protein; unnamed protein product; chrom 17
RC_H93021_at	chrom 2 ; XM_004890 peptidylprolyl isomerase A (cyclophilin A)
RC_AA427737_at	no homology
RC_AA417078_at	chrom 7q31; AF017104 clone
M29873_s_at	cytochrome P450-IIB (hIIB3) ; 19q13.1-q13.2

wherein the notation refers to Accession No. in the database UniGene (Build 18).

56. The method according to any of claims 43-49, wherein the second gene group are selected individually from genes comprising a sequence as identified below

RC_AA609013_s_at	microsomal dipeptidase (also on 6.8k); chrom 16
RC_AA232508_at	CGI-89 protein; unnamed protein product; hypothetical protein
RC_AA428964_at	serine protease-like protease; serine protease homolog=NES1; normal epithelial cell-specific 1
RC_AA075642_at	gp-340 variant protein; DMBT1/8kb.2 protein
RC_AA007218_at	chrom 13 no homology
RC_N33920_at	ubiquitin-like protein FAT10; diubiquitin; dJ271M21.6 (Diubiquitin); chrom 6
RC_N71781_at	KIAA1199 protein, chrom 15
RC_R67275_s_at	alpha-1 (type XI) collagen precursor; collagen, type XI, alpha 1; collagen type XI alpha-1 isoform A; chrom 1
RC_W80763_at	hypothetical protein; chrom 17
RC_AA034499_s_at	ZNF198 protein; zinc finger protein; FIM protein; Cys-rich protein; zinc finger protein 198; chrom 13
RC_AA035482_at	chrom 5; AK022505 clone; CalcineurinB (weakly similar)
RC_AA024482_at	hypothetical protein; unnamed protein product; chrom 17
RC_H93021_at	chrom 2 ; XM_004890 peptidylprolyl isomerase A (cyclophilin A)
RC_AA427737_at	no homology
RC_AA417078_at	chrom 7q31; AF017104 clone
M29873_s_at	cytochrome P450-IIB (hIIB3) ; 19q13.1-q13.2

wherein the notation refers to Accession No. in the database UniGene (Build 18).

57. The method according to any of claims 43-49, wherein the second gene group comprises a sequence as identified below

RC_W80763_at		Hypothetical protein; chrom 17

wherein the notation refers to Accession No. in the database UniGene (Build 18).

58. The method according to any of the preceding claims 43-57, wherein the expression level of at least two genes from the first gene group are determined

59. The method according to any of the preceding claims 43-58, wherein the expression level of at least two genes from the second gene group are determined.

60. A method of determining an expression pattern of a colon cell sample independent of the proportion of submucosal, muscle, or connective tissue cells present, comprising:

determining the expression of one or more genes in a sample comprising cells, wherein the one or more genes exclude genes which are expressed in the submucosal, muscle, or connective tissue, whereby a pattern of expression is formed for the sample which is independent of the proportion of submucosal, muscle, or connective tissue cells in the sample.

61. The method according to claim 60, comprising determining the expression level of one or more genes in the sample comprising predominantly submucosal, muscle, and connective tissue cells, obtaining a second pattern, subtracting said second pattern from the expression pattern of the colon and/or rectum cell sample, forming a third pattern of expression, said third pattern of expression reflecting expression of the colon cells independent of the proportion of submucosal, muscle, and connective tissue cells present in the sample.

62. A method of determining the presence or absence of a biological condition in human colon and/or rectum tissue comprising,

collecting a sample comprising cells from the tissue,

determining an expression pattern of the cells as defined in any of claims 43-61,

correlating the determined expression pattern to a standard pattern,

determining the presence or absence of the biological condition of said tissue.

63. A method for determining the stage of a biological condition in animal tissue, comprising

collecting a sample comprising cells from the tissue,

determining an expression pattern of the cells as defined in any of claims 43-61,

correlating the determined expression pattern to a standard pattern,

determining the stage of the biological condition is said tissue.

64. A method for reducing cell tumorigenicity of a cell, said method comprising

contacting a tumor cell with at least one peptide expressed by at least one gene selected from genes being expressed in an at least two-fold higher in normal cells than the amount expressed in said tumor cell.

65. The method according to claim 64, wherein the at least one gene is selected individually from genes comprising a sequence as identified below

RC_H04768_at	chrom 15 no homology
RC_Z39652_at	Y14593 APM-1 gene adipocyte-specific secretory protein; chrom 1q21.3-q23
RC_H30270_at	chrom 18 PAAAA in colon & bladder no homology
RC_T47089_s_at	tenascin-X; tenascin-X precursor; unidenti

	fied protein
RC_W31906_at	secretagogin; dJ501N12.8 (putative protein) chrom 6
RC_AA279803_at	chrom 2 no homology
RC_R01646_at	chrom 13q32.1-33.3 ; AL159152 ; homology to mouse Pcbp1 - poly(rC)-binding protein 1
AA319615_at	secretory carrier membrane protein; secretory carrier membrane protein 2; chrom 15

wherein the notation refers to Accession No. in the database UniGene (Build 18).

66. The method according to claim 64 or 65, wherein the tumor cell is contacted with at least two different peptides.

67. A method for reducing cell tumorigenicity of a cell, said method comprising

obtaining at least one gene selected from genes being expressed in an at least two-fold higher in normal cells than the amount expressed in said tumor cell,

introducing said at least one gene into the tumor cell in a manner allowing expression of said gene(s).

68. The method according to claim 67, where the at least one gene is selected individually from genes comprising a sequence as identified below

RC_H04768_at	chrom 15 no homology
RC_Z39652_at	Y14593 APM-1 gene adipocyte-specific secretory protein; chrom 1q21.3-q23
RC_H30270_at	chrom 18 PAAAA in colon & bladder no homology
RC_T47089_s_at	tenascin-X; tenascin-X precursor; unidentified protein
RC_W31906_at	secretagogin; dJ501N12.8 (putative protein) chrom 6
RC_AA279803_at	chrom 2 no homology
RC_R01646_at	chrom 13q32.1-33.3 ; AL159152 ; homology to mouse Pcbp1 - poly(rC)-binding protein 1
AA319615_at	secretory carrier membrane protein; secretory carrier membrane protein 2; chrom 15

wherein the notation refers to Accession No. in the database UniGene (Build 18).

69. The method according to claim 67 or 68, wherein at least two different genes are introduced into the tumor cell.

70. A method for reducing cell tumorigenicity of a cell, said method comprising

obtaining at least one nucleotide probe capable of hybridising with at least one gene of a tumor cell, said at least one gene being selected from genes being expressed in an amount at least one-fold lower in normal cells than the amount expressed in said tumor cell, and

introducing said at least one nucleotide probe into the tumor cell in a manner allowing the probe to hybridise to the at least one gene, thereby inhibiting expression of said at least one gene.

71. The method according to claim 70, wherein the nucleotide probe is selected from probes capable of hybridising to a nucleotide sequence comprising a sequence as identified below

RC_AA609013_s_at	APPPP	microsomal dipeptidase (also on 6.8k); chrom 16
RC_AA232508_at	APPPP	CGI-89 protein; unnamed protein product; hypothetical protein
RC_AA428964_at	APPPP	serine protease-like protease; serine protease homolog=NES1; normal epithelial cell-specific 1
RC_T52813_s_at	APPPP	dJ28O10.2 (G0S2 (PUTATIVE LYMPHOCYTE G0/G1 SWITCH PROTEIN 2; chrom 1
RC_AA075642_at	APPPP	gp-340 variant protein; DMBT1/8kb.2 protein
RC_AA007218_at	APPPP	chrom 13 no homology
RC_N33920_at	APPPP	ubiquitin-like protein FAT10; diubiquitin; dJ271M21.6 (Diubiquitin); chrom 6
RC_N71781_at	APPPP	KIAA1199 protein, chrom 15

RC_R67275_s_at	APPPP	alpha-1 (type XI) collagen precursor; collagen, type XI, alpha 1; collagen type XI alpha-1 isoform A; chrom 1
RC_W80763_at	APPPP	hypothetical protein; chrom 17
RC_AA443793_at	APPPP	chrom 7p22 AC006028 BAC clone
RC_AA034499_s_at	APPPP	ZNF198 protein; zinc finger protein; FIM protein; Cys-rich protein; zinc finger protein 198; chrom 13
RC_AA035482_at	APPPP	chrom 5; AK022505 clone; CalcineurinB (weakly similar)
RC_AA024482_at	APPPP	hypothetical protein; unnamed protein product; chrom 17
RC_H93021_at	APPPP	chrom 2 ; XM_004890 peptidylprolyl isomerase A (cyclophilin A)
RC_AA427737_at	APPPP	no homology
RC_AA417078_at	APPPP	chrom 7q31; AF017104 clone
M29873_s_at	APPPP	cytochrome P450-IIB (hIIB3) ; 19q13.1-q13.2
RC_H27498_f_at	AAPPP	
RC_T92363_s_at	AAPPP	
RC_N89910_at	AAAPP	
RC_W60516_at	AAAPP	
RC_AA219699_at	AAAPP	
RC_AA449450_at	AAAPP	

wherein the notation refers to Accession No. in the database UniGene (Build 18).

72. The method according to claim 70 or 71, wherein at least two different genes are introduced into the tumor cell.

73. A method for producing antibodies against an expression product of a cell from a biological tissue, said method comprising the steps of

obtaining expression product(s) from at least one gene said gene being expressed as defined in any of claims 27-37,

immunising a mammal with said expression product(s) obtaining antibodies against the expression product.

74. A pharmaceutical composition for the treatment of a biological condition comprising at least one antibody produced as described in claim 73.

75. A vaccine for the prophylaxis or treatment of a biological condition comprising at least one expression product from at least one gene said gene being expressed as defined in any of claims 27-37.

76. The use of a method as defined in any of claims 1-63 for producing an assay for diagnosing a biological condition in animal tissue.

77. The use of a peptide as defined in any of claims 64-66 for preparation of a pharmaceutical composition for the treatment of a biological condition in animal tissue.

78. The use of a gene as defined in any of claims 67-69 for preparation of a pharmaceutical composition for the treatment of a biological condition in animal tissue.

79. The use of a probe as defined in any of claims 70-72 for preparation of a pharmaceutical composition for the treatment of a biological condition in animal tissue.

80. An assay for determining the presence or absence of a biological condition in animal tissue, comprising

at least one first marker capable of detecting a first expression level of at least one gene from a first gene group, wherein the gene from the first gene group is selected from genes expressed in normal tissue cells in an amount higher than expression in biological condition cells,

at least one second marker capable of detecting a second expression level of at least one gene from a second gene group, wherein the second gene group is selected from genes expressed in normal tissue cells in an amount lower than expression in biological condition cells.

81. The assay according to claim 80, wherein the marker is a nucleotide probe.

82. The assay according to claim 80, wherein the marker is an antibody.

83. The assay according to claim 80, wherein the genes are as defined in any of claims 11-18, 34-37, and 39-42.

84. An assay for determining an expression pattern of a colon and/or rectum cell, comprising at least a first marker and a second marker, wherein the first marker is capable of detecting a gene from a first gene group as defined in claim 43, and the second marker is capable of detecting a gene from a second gene group as defined in claim 43.

85. The assay according to claim 84, wherein the first marker is capable of detecting one gene as identified in Table I, and the second marker is capable of detecting another gene as identified in Table I.

86. The assay according to claim 85, comprising at least two markers for each gene group,

correlating the first expression level and the second expression level to a standard level of the assessed genes to determine the presence or absence of a biological condition in the animal tissue.

87. The assay according to claim 86, wherein the marker is a nucleotide probe

88. The assay according to claim 86, wherein the marker is an antibody.

89. A method for identifying a tissue sample as colo-rectal, comprising subjecting the tissue to a method as identified in any of claims 43-61, determining expression patterns and comparing the expression patterns determined with expression patterns from colo-rectal tissue.

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